

# Phylogeny and evolution of male genitalia within the praying mantis genus *Tenodera* (Mantodea : Mantidae)

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**Abstract.** The mantis genus *Tenodera* is composed of several species distributed across Africa, Asia and Australasia, along with recent human introductions to North America. Species of the genus are morphologically similar and utilise equivalent habitats across their distribution. Relationships among these species and the morphological characters used to diagnose them have never been formally tested, leaving authors to disagree as to the species composition of *Tenodera*. With DNA sequence data from five molecular loci and morphological characters from male genitalia, we reconstructed the phylogeny of *Tenodera* using multiple optimality criteria. All included species were found to be monophyletic in analyses of the combined data. *Tenodera sinensis* and *T. bokiana* were both supported as distinct species recovered in separate clades, resolving confusion as to their placement and classification. Our analysis identified a previously undescribed species of *Tenodera* collected in India, recovered as sister to *T. aridifolia* and *T. sinensis*, and exhibiting distinct male genital morphology. In light of the phylogeny, we characterise for the first time, and investigate the evolution of, the male genitalia, which allowed us to discover several transitions in structural forms. We also consider the connection of these transitions to sexual cannibalism and how this behaviour may have led to rapid evolution of the male genitalia.

**Additional keywords:** cannibalism, Chinese mantis, genital morphology.

## Introduction

*Tenodera* is one of the most recognised mantis genera in the world. It comprises 15 species (Ehrmann 2002; Otte and Spearman 2005), including the ‘Chinese mantis’ *T. sinensis*, which is the most widespread and abundant of all temperate zone mantis species (Hurd 1999). This cosmopolitan genus has its greatest diversity centred in Australasia, with species typically inhabiting grasslands and forest edges across both temperate and tropical climates (Hurd 1999). Like the Chinese mantis, other species in the genus are commonly encountered outside their natural habitats, often in urban and suburban areas. Most of what we know about *Tenodera* biology is derived from the temperate species *T. aridifolia* (Hurd and Eisenberg 1984; Hurd 1999), but it appears that many aspects of behaviour, morphology, physiology and ecology are similar across all species. For instance, *T. aridifolia*, and presumably other temperate species, are univoltine and will mate in late summer such that the eggs will over-winter (Hurd 1999). Species of *Tenodera* are ambush hunters with a list of prey that suggests they are generalist or opportunistic feeders (Svenson and Whiting 2004). Their prey consists mainly of other insects, though some species have been known to catch small frogs and hummingbirds (Milne and Milne 1995).

For a mantis genus, *Tenodera* is relatively well known, but the taxonomy remains poorly understood. Since the description of the genus in 1838 by Burmeister, more than 30 species have

been proposed, but the group has never been subjected to a comprehensive taxonomic analysis. In their catalogues of Mantodea, Ehrmann (2002) recognised 13 species and 2 subspecies for the genus, whereas Otte and Spearman (2005) recognised 20 species and 13 subspecies. The incongruence between these two classification schemes illustrates the lack of clear diagnostic characters for delimiting species within the genus. The Chinese mantis, *T. sinensis*, was considered a valid species by Giglio-Tos (1927) and Ehrmann (2002) but as a subspecies of *T. aridifolia* by Otte and Spearman (2005) based only on the size difference between the two. Observations of size variation within and among species of *Tenodera* (Dusse and Hurd 1997) raise questions about the validity of species diagnosed by size alone. Another potential problem in species diagnoses lies in the reliance on coloration. All species in the genus appear to exhibit similar coloration patterns, which are usually a tan/brown cuticle and the tegmina with a tan discoidal and anal region while the costal region is noticeably green (Milne and Milne 1995). Based on the coloration of forelegs, *T. bokiana* has been treated either as a valid species (Giglio-Tos 1927), a subspecies of *T. superstitionosa* (Otte and Spearman 2005) or a synonym of *T. superstitionosa* (Ehrmann 2002), though it is unclear whether coloration is a diagnostic character in this mantis lineage. Therefore, a more reliable character system needs to be explored to better understand the systematics of *Tenodera*.

In this study, we investigate the taxonomic utility of two systems in particular: molecular loci and male genitalia. We reconstruct a phylogeny of the genus based on these characters to assess the current species boundaries. Specifically, we perform an in-depth examination of the taxonomic utility of male genitalia, which are generally very useful in diagnosing species in insect systematics (Tuxen 1956) including Mantodea (Klass 1997), though they have not been previously explored within *Tenodera* systematics. Our preliminary investigation suggests that male genitalia of *Tenodera* may provide species-specific diagnostic characters that can help resolve the taxonomic confusion, as has been found in other mantis genera (Holwell 2008).

The study of male genitalia in *Tenodera* presents a unique opportunity to understand how this structure might have evolved in mantises. The current paradigm in the study of genital evolution is that male genitalia are sexually selected (Eberhard 1985; Arnqvist 1998; Hosken and Stockley 2004). *Tenodera* species typically mate multiple times (Bartley 1982; Hurd *et al.* 1994) and there is a direct physical contact between male and female genital organs, which satisfy the criteria for which sexual selection can act upon genital evolution (Eberhard 1985). At the same time, there may be additional selective pressure influencing the genital evolution in light of the well known mating biology of mantises: female-on-male cannibalism (Birkhead *et al.* 1988; Hurd *et al.* 1994; Maxwell 1999). In *Tenodera*, females gain valuable nutritional resources through cannibalism (Hurd *et al.* 1994), but it is not clear whether males gain any benefit through complicity (Birkhead *et al.* 1988; Lelito and Brown 2006). Lelito and Brown (2006) recently demonstrated that males of *T. sinensis* behaved in a manner indicative of risk avoidance during mating, thereby concluding that there was no strong evidence for male complicity in these mantises and suggested a possible role of sexual conflict in this system. Working with a framework of cannibalistic spiders, Miller (2007) demonstrated that male sacrifice behaviour was phylogenetically correlated with another phenomenon known as genital mutilation. Based on this observation, he further argued that, when sexual cannibalism is present, male genitalia may be expected to evolve to break or become disfigured during copulation, thereby negatively affecting the future reproductive potential of the female (Miller 2007). This type of genital mutilation has never been reported in insects to our knowledge, but genital damage, in which male genitalia would injure female genital tracts to increase the fitness of the male by reducing the risk of sperm competition, has been reported in beetles (Crudgington and Siva-Jothy 2000). If genital damage prevails in *Tenodera* mating systems, it may be possible to discover male genital structures consistent with this prediction and such structures would be under high selective pressure.

In this study, we aim to address the following questions using the first explicit phylogeny of *Tenodera*. (1) Is *Tenodera* monophyletic and what are the relationships among the sampled species? (2) Do male genital structures provide reliable diagnostic character systems, and are phylogenetic hypotheses based on these characters congruent with those derived from molecular data? (3) How did the male genitalia of *Tenodera* evolve in light of the phylogeny and in the context of sexual cannibalism?

## Materials and methods

### *Taxon and character sampling*

We sampled 32 *Tenodera* exemplars representing seven species from six geographic regions (Table 1) for phylogenetic analyses. The identification of these specimens was determined using the key in Giglio-Tos (1927) while cross-referencing with both an India-focussed key by Mukherjee *et al.* (1995) and an Africa-based key by Kaltenbach (1996). We found a unique specimen from India that is similar to *T. aridifolia*, but differs in the morphology of the genitalia. Nine outgroup taxa were selected from sister genera within the subfamily Mantinae (*sensu* Ehrmann 2002) based on previous molecular-based analyses of Mantodea (Svenson and Whiting 2004; Yager and Svenson 2008). Voucher specimens for morphology and molecular data are deposited at the M. L. Bean Museum, Brigham Young University. There are multiple ways that species can be delimited using phylogenetic and other methods (reviewed in Sites and Marshall 2003). We adhered to the phylogenetic species concept *sensu* Nixon and Wheeler (1990), where species are diagnosed with unique combinations of characters and their boundaries demarcated via the reconstructed phylogeny.

To reconstruct the phylogeny of *Tenodera*, we targeted a total of five loci, including two nuclear protein-coding genes, histone-III (H3, ~352 bp) and wingless (Wg, ~401 bp), and three mitochondrial genes, large rRNA (16S, ~470 bp), small rRNA (12S, ~345 bp), and cytochrome oxidase II (COII, ~690 bp). Genomic DNA was extracted from thoracic muscle tissue using the DNeasy tissue kit (Qiagen, Valencia, CA). We followed the PCR protocols and primers described in Svenson and Whiting (2004). Sequencing was performed using ABI BigDye version 3 dye terminator chemistry and then fractionated on an ABI 3730xl capillary sequencer (Applied Biosystems Inc., Foster City, CA). Sequence data were imported into Sequencher 4.0 (Genecodes 1999) for nucleotide editing and contig assembly.

### *Phylogenetic analyses*

We manually aligned protein-coding genes based on the conservation of reading frames while ribosomal genes were partitioned into conserved and non-conserved regions and assembled separately in Sequencher 4.0. The ribosomal gene partitions were then exported and aligned using the program MAFFT ver. 5.8 (Katoh *et al.* 2002; Katoh *et al.* 2005) using the L-INS-i algorithm with 'maxiterate' set to 1000. The final molecular matrix contained 2270 aligned nucleotide characters of which 339 characters were phylogenetically informative. The dataset was analysed in three phylogenetic inference frameworks: maximum parsimony (MP), partitioned maximum likelihood (PML) and mixed model Bayesian (MMB). The MP search was performed with 5000 random additions with TBR in PAUP\* 4.0b10 (Swofford 2002). To assess nodal support, we calculated nonparametric bootstrap values derived from 500 replicates. For PML, 10 independent runs were performed, each with 5000 replicates in Treefinder (Jobb 2006) after determining the following best-fit model for each locus under the Akaike Information Criteria, implemented in ModelTest 3.7 (Posada and Crandall 1998): 12S = HKY+I+G; 16S = TrN+I+G; COII = GTR+I+G; H3 = TrN+I; Wg = TrN+I. In addition, we

**Table 1.** List of the mantis specimens used in this study with collecting locality data, voucher information and GENBANK accession numbers  
Absent data are listed as NA

Species	Collecting site	Voucher number	GENBANK accession numbers
			12S/16S/Cytochrome Oxidase II/Histone 3/Wingless
<i>Heirodula</i>			
<i>H. sp.</i> ♀		MN019	GU064647/GU064684/GU064722/GU064763/GU064803
<i>H. schultzei</i> ♂		MN044	GU064650/GU064687/GU064725/GU064766/GU064806
<i>Tamolanica</i>			
<i>Ta. tamolana</i> ♂		MN020	GU064648/GU064685/GU064723/GU064764/GU064804
<i>Sphodromantis</i>			
<i>S. viridis</i> ♀		MN013	GU064645/GU064682/GU064720/GU064761/GU064801
<i>S. lineola</i> ♀		MN015	GU064646/GU064683/GU064721/GU064762/GU064802
<i>Polyspilota</i>			
<i>P. aerinosa</i> ♀		MN167	GU064651/GU064688/GU064726/GU064767/GU064807
<i>P. aeruginosa</i> ♂		MN248	GU064653/GU064690/GU064728/GU064769/GU064809
<i>Plistospilota</i>			
<i>Pl. guineensis</i> ♂		MN236	GU064652/GU064689/GU064727/GU064768/GU064808
<i>Prohierodula</i>			
<i>Pr. ornatipennis</i> ♂		MN249	GU064654/GU064691/GU064729/GU064770/GU064810
<i>Tenodera</i>			
<i>T. australasiae</i> ♂	Australia, Queensland	MN025	GU064649/GU064686/GU064724/GU064765/GU064805
<i>T. australasiae</i> ♂	Australia, Northern Territory	MN505	GU064660/GU064697/GU064735/GU064776/GU064816
<i>T. sp. nov.</i> ♂	India, Kerala	MN519	GU064674/GU064710/GU064749/NA/GU064830
<i>T. sinensis</i> ♂	USA	MN520	GU064675/GU064711/GU064750/GU064790/GU064831
<i>T. sinensis</i> ♀	USA, NC, Wake County	MN521	GU064676/GU064712/GU064751/GU064791/GU064832
<i>T. sinensis</i> ♀	USA, NC, Wake County	MN522	GU064677/GU064713/GU064752/GU064792/GU064833
<i>T. sinensis</i> ♀	USA	MN002	GU064644/GU064681/GU064719/GU064760/GU064800
<i>T. sinensis</i> ♂	Japan	MN525	GU064680/NA/GU064755/GU064795/NA
<i>T. aridifolia</i> ♂	India, Meghalaya	MN517	GU064672/GU064708/GU064747/GU064788/GU064828
<i>T. aridifolia</i> ♂	India, Meghalaya	MN518	GU064673/GU064709/GU064748/GU064789/GU064829
<i>T. aridifolia</i> ♂	Borneo	MN528	NA/GU064716/GU064757/GU064797/GU064837
<i>T. aridifolia</i> ♂	Borneo	MN527	NA/GU064715/GU064756/GU064796/GU064836
<i>T. aridifolia</i> ♂	Borneo	MN530	NA/GU064718/GU064759/GU064799/GU064839
<i>T. costalis</i> ♀	Papua New Guinea, Chimbu Province	MN512	GU064667/NA/GU064742/GU064783/GU064823
<i>T. costalis</i> ♀	Papua New Guinea, Chimbu Province	MN511	GU064666/GU064703/GU064741/GU064782/GU064822
<i>T. costalis</i> ♀	Papua New Guinea, Eastern Highlands Province	MN506	GU064661/GU064698/GU064736/GU064777/GU064817
<i>T. costalis</i> ♀	Papua New Guinea, Eastern Highlands Province	MN501	GU064656/GU064693/GU064731/GU064772/GU064812
<i>T. costalis</i> ♀	Papua New Guinea, Eastern Highlands Province	MN507	GU064662/GU064699/GU064737/GU064778/GU064818
<i>T. costalis</i> ♀	Papua New Guinea, Morobe Province	MN510	GU064665/GU064702/GU064740/GU064781/GU064821
<i>T. costalis</i> ♂	Papua New Guinea, Morobe Province	MN508	GU064663/GU064700/GU064738/GU064779/GU064819
<i>T. costalis</i> ♀	Papua New Guinea, Morobe Province	MN509	GU064664/GU064701/GU064739/GU064780/GU064820
<i>T. superstiosa</i> ♂	Zambia, Copperbelt Province	MN524	GU064679/GU064714/GU064754/GU064794/GU064835
<i>T. superstiosa</i> ♀	Republic of South Africa, Kruger National Park	MN514	GU064669/GU064705/GU064744/GU064785/GU064825
<i>T. superstiosa</i> ♀	Republic of South Africa, Kruger National Park	MN515	GU064670/GU064706/GU064745/GU064786/GU064826
<i>T. superstiosa</i> ♀	Africa	MN523	GU064678/NA/GU064753/GU064793/GU064834
<i>T. bokiana</i> ♂	Papua New Guinea, Eastern Highlands Province	MN513	GU064668/GU064704/GU064743/GU064784/GU064824
<i>T. bokiana</i> ♀	Australia, Queensland	MN500	GU064655/GU064692/GU064730/GU064771/GU064811
<i>T. bokiana</i> ♀	Papua New Guinea, Central Province	MN504	GU064659/GU064696/GU064734/GU064775/GU064815
<i>T. bokiana</i> ♀	Papua New Guinea, Sandaun Province	MN503	GU064658/GU064695/GU064733/GU064774/GU064814
<i>T. bokiana</i> ♀	Papua New Guinea, Sandaun Province	MN502	GU064657/GU064694/GU064732/GU064773/GU064813
<i>T. bokiana</i> ♂	Malaysia, Sabah	MN516	GU064671/GU064707/GU064746/GU064787/GU064827
<i>T. bokiana</i> ♂	Borneo	MN529	NA/GU064717/GU064758/GU064798/GU064838

calculated partitioned likelihood nonparametric bootstrap values consisting of 500 replicates. The MMB analysis was performed by running four independent runs with four chains (1 cold and 3 hot) for 20 million generations, sampling every 2000 generations in MrBayes ver. 3.1.2 (Ronquist and Huelsenbeck 2003). The HKY+I+G model was applied to 12S, 16S, H3 and Wg, and the GTR+I+G model was applied to the COII partition in

the MMB analysis. To ensure convergence between runs, log-likelihood values were monitored in Tracer ver. 1.3 (Rambaut and Drummond 2003) and all sampled generations (2 million generations) before stationarity were discarded as burn-in. The results of the MMB analyses were summarised as a 50% majority-rule consensus tree (Huelsenbeck and Imennov 2002; Huelsenbeck *et al.* 2002).

### Male genitalia character evolution

To examine taxonomic utility of male genitalia in *Tenodera*, we characterised genital morphology of each species in detail. Several species were represented by multiple specimens to detect intraspecific variation (Table 1). To extract the phallic complex, terminal abdominal segments were dissected and placed in a hot weak KOH solution for 40 min to dissolve muscle tissue. Cleared genital structures were illustrated with the aid of a camera lucida, and were also digitally photographed using Olympus SZX12 and Olympus MicroSuite software (version B3SV; Olympus, Tokyo). We followed the terminology of Tuxen (1956) and Klass (1997) in describing genital structures.

The search for diagnostic characters was achieved by coding 24 discrete genital characters. These characters were coded as unordered and equally weighted in WinClada ver. 1.00.08 (Nixon 2002). To examine phylogenetic utility of male genitalia, we reconstructed a phylogeny based only on genital characters and compared it with the molecular phylogeny. The morphology matrix was analysed in NONA (Goloboff 1995) using the following commands: rs 0; hold 1000; mult\*50. To test whether there were conflicting signals between molecular and genital characters, we combined both datasets and analysed simultaneously in a parsimony framework in TNT (Goloboff *et al.* 2003) using the search methods including parsimony ratchet, tree fusing and tree drift, while treating gaps as missing. We treated male genital characters as missing data for female specimens included in the molecular dataset. We calculated nonparametric bootstrap values (Felsenstein 1985) and partitioned Bremer support values using TreeRot v2c (Sorenson 1999) in conjunction with PAUP, though we increased the random additions per nodal constraint search from 10 to 40 to more thoroughly estimate Bremer values. Finally, to explore character evolution of male genitalia in *Tenodera*, we optimised genital characters onto the cladograms based on morphology only as well as the combined dataset using parsimony methods (ACCTRAN, DELTRAN, unambiguous) in WinClada (Nixon 2002).

## Results

### Phylogenetic analyses

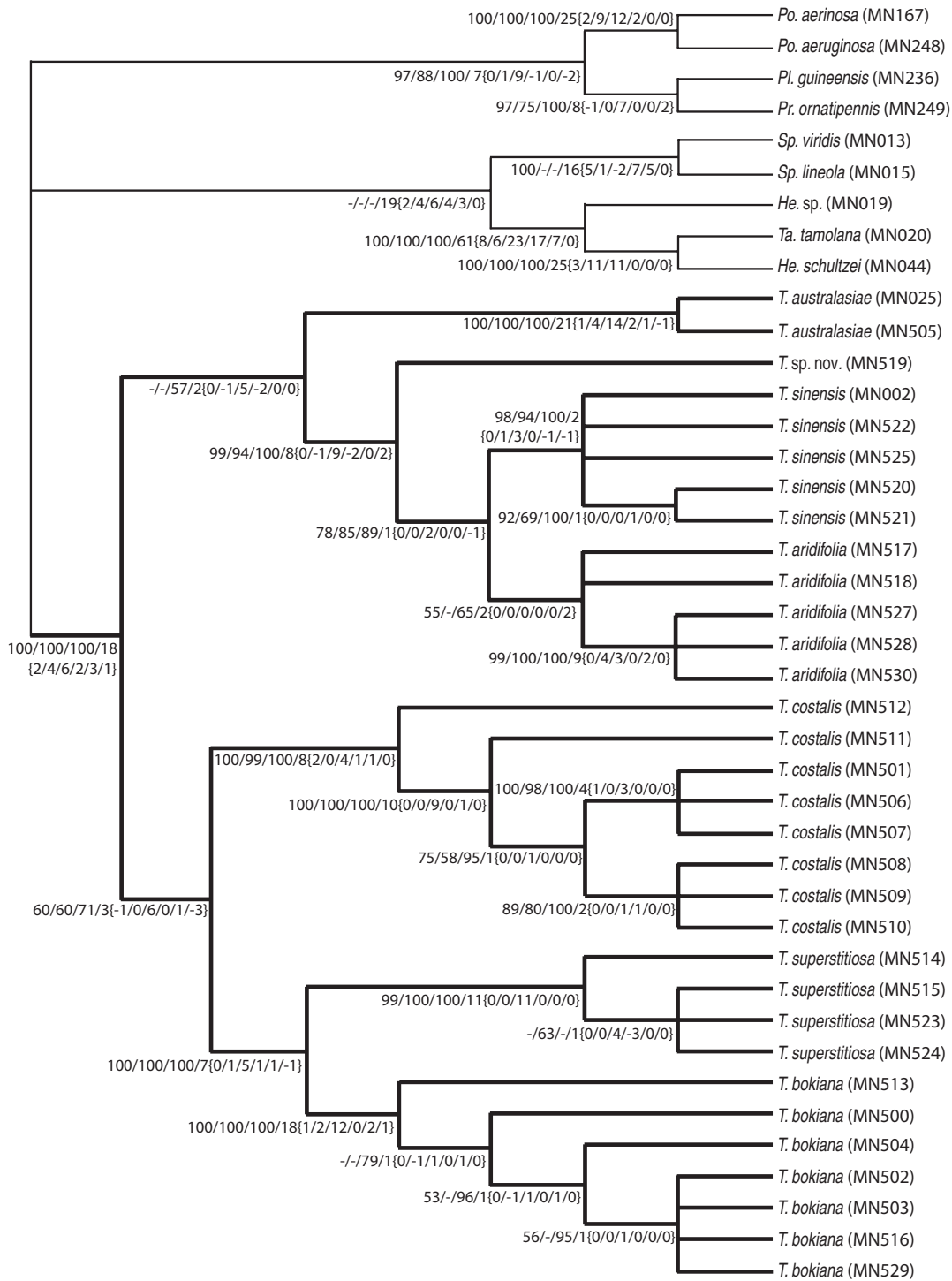
Based on the molecular dataset, a monophyletic *Tenodera* with strong nodal support values was recovered in MP (20 MPTs,  $L=973$ ,  $CI=0.62$ ,  $RI=0.81$ ), PML (likelihood = -9690.4553) and MMB (mean likelihood = -8421.141) analyses, with nearly congruent topologies (Fig. 1). The included genera from Polyspilotini were consistently recovered as sister to *Tenodera*. Intraspecific relationships were variable across different analyses, but the monophyly of individual *Tenodera* species, including *T. sinensis* and *T. bokiana*, was consistently recovered. The position of *T. australasiae* was unstable and was recovered in different positions by different inference methods. For instance, its position was unresolved in the MP analysis, sister to (*T. costalis* (*T. superstitiosa* + *T. bokiana*)) clade in the PML analysis, and sister to (*T. aridifolia* + *T. sinensis*) clade in the MMB analysis. Other than the placement of *T. australasiae*, the species of *Tenodera* were recovered in two major clades, one

consisting of *T. sinensis*, *T. aridifolia* and *T. sp. nov.* and another consisting of *T. costalis*, *T. superstitiosa* and *T. bokiana*, with the only differences occurring with the placement of individuals within species. Within the *T. costalis* clade, we found that one individual (MN512) was placed basally to the rest of the clade, separated by a long branch (Fig. 2). This individual was a female specimen that was identified as *T. costalis* based on a few diagnostic characters, but it is possible that this specimen does not belong to *T. costalis* as it is often very difficult to identify species of mantises from female specimens. Interestingly, the unique Indian exemplar similar to *T. aridifolia* was consistently placed sister to the (*T. aridifolia* + *T. sinensis*) clade. After a close examination of the male genital morphology (Fig. 4G) and the genetic distance of this species to the (*T. aridifolia* + *T. sinensis*) clade (Fig. 2), we determined this specimen to be an undescribed species. The analysis based on male genital structures recovered 10 most parsimonious trees ( $L=48$ ;  $CI=0.77$ ;  $RI=0.79$ ) from which a strict consensus collapsed five nodes, which did not conflict with the molecular analysis (Fig. 3). A monophyletic *Tenodera* was strongly supported, although the ingroup relationships were largely unresolved, except for a trichotomy consisting of *T. aridifolia*, *T. sinensis* and *T. sp. nov.*

The parsimony analysis of the combined data recovered a topology nearly identical to the MMB tree, although the intraspecific relationships were slightly different (12 MPTs,  $L=1026$ ,  $CI=0.63$ ,  $RI=0.81$ ). The strict consensus tree placed the undescribed Indian species as sister to (*T. aridifolia* + *T. sinensis*) with good support, while *T. australasiae* was recovered as the sister group to the entire clade (Fig. 1). Although the MP analysis of the molecular dataset alone was unable to recover a monophyletic *T. aridifolia*, the inclusion of genital characters provided additional signal that grouped the specimens into a single clade.

The Bremer support values reported on the combined analysis (Fig. 1) indicate that some of the morphological and molecular data do not offer strong phylogenetic signal. We find that 57% of the signal comes from COII, 15% from 16S, 10% from H3, 9.5% from Wg and 8.5% from 12S. The data obtained from our morphology matrix do not provide sufficient signal to infer relationships. We find that species within *Tenodera* are not always monophyletic in phylogenies reconstructed using single genes and that *T. aridifolia* is never recovered as monophyletic. Since support for *T. aridifolia* and *T. sinensis* as monophyletic species is only found with the addition of the morphological data, this may suggest that these two are a single species with variable morphology. The Indian species is recovered as monophyletic and sister to *T. aridifolia* and *T. sinensis* when reconstructing phylogenies using COII and the morphological data. Since a high level of signal comes from COII in addition to the strong support from the other analyses (Fig. 1), this supports a possible undescribed species. We suggest that the negative Bremer support for morphology found in some taxa (i.e. *T. sinensis* and *T. australasiae*; Fig. 1) may be explained by internal morphological character conflict, meaning the molecular signal for these taxa is more robust than the signal from morphology.



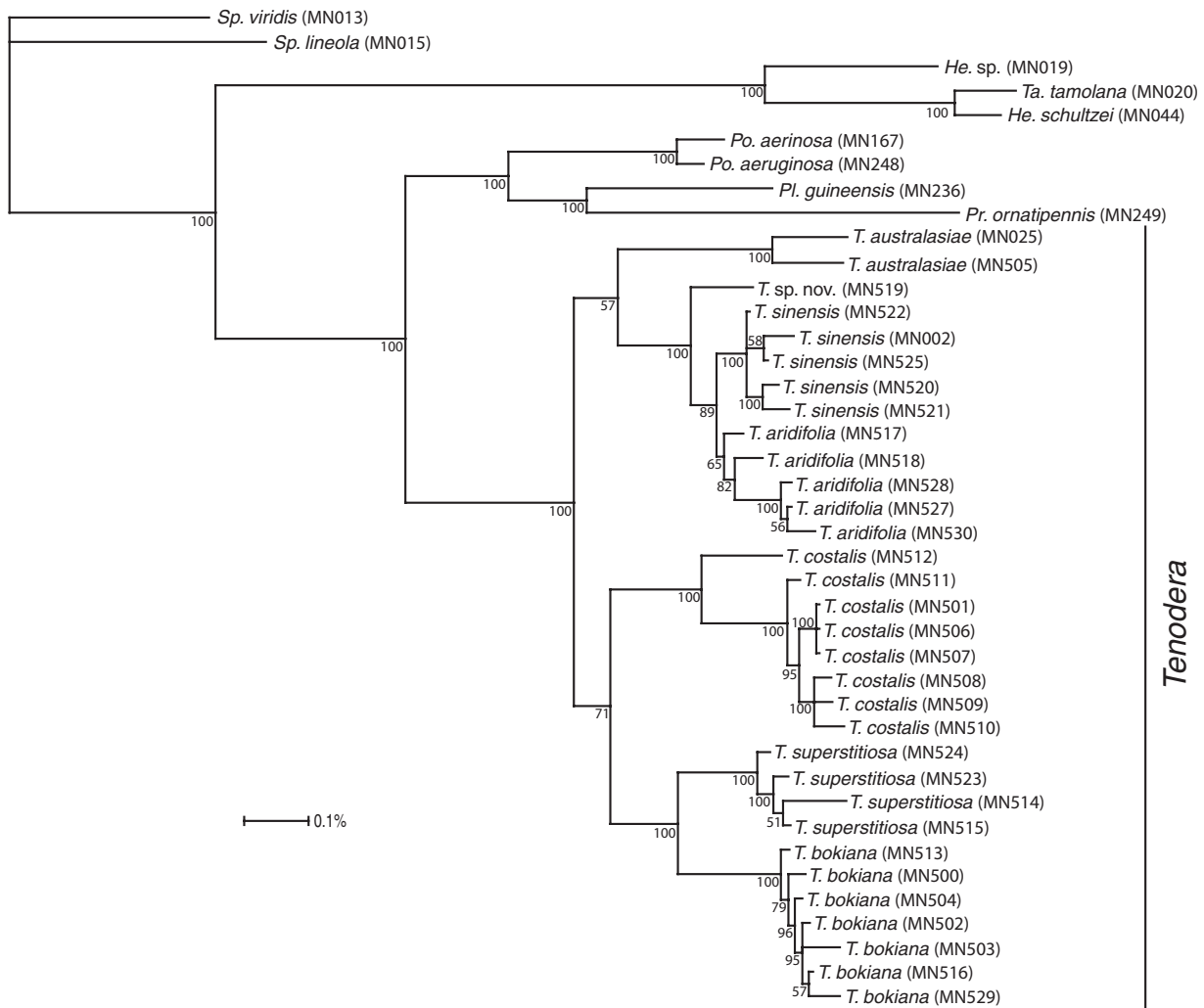


**Fig. 1.** Strict consensus of the maximum parsimony (MP) analysis of combined datasets with gaps treated as missing data. Nodal support is shown with likelihood bootstrap, Nonparametric bootstrap, posterior probability, total Bremer followed by the partitioned Bremer support values for {12S, 16S, COII, H3, Wingless, and Morphology}.

*Male genital character evolution*

From the 24 characters (Appendices 1 and 2) coded from the male genitalia of *Tenodera*, we identified four taxonomically diagnostic structures for the genus (Fig. 4): the acutolobus

shape being apically bulbous and smooth with a spine or a rough blade shape; the hypophallus orientation measured in relation to the base of the left epiphallus and what shape the distal portion takes; the width of the pseudophallus in relation to

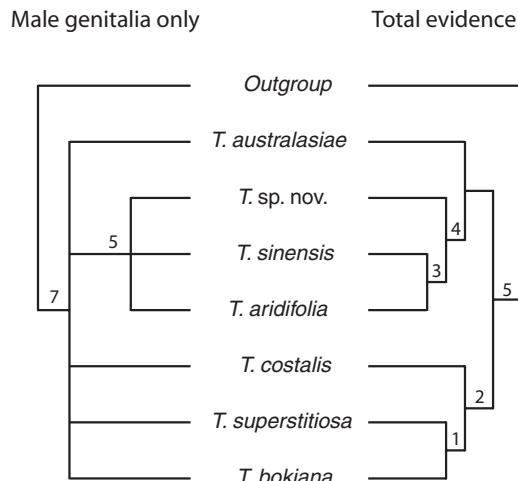


**Fig. 2.** Majority rule consensus phylogram from the mixed-model Bayesian (MMB) analysis. Numbers above the nodes represent posterior probability. The scale bar represents 0.1% sequence divergence.

the stalk and the orientation from that stalk; and the shape of the titillator. The variation of these four structures was also informative in species diagnosis (Fig. 5).

We identified several synapomorphies based on a combined dataset under both ACCTRAN and DELTRAN optimisations. Under ACCTRAN, we found the following genital characters support the monophyly of the genus: the orientation of the hypophallus (Char 7, state 2), the pseudophallus being bent (Char 14, state 1), the size of the small lobe on the right epiphallus (Char 18, state 1), and the curvature of the titillator (Char 20, state 2). Other combinations of characters supported the monophyly of the genus under DELTRAN optimisation: the shape of the acutolobus (Char 2, state 1), the hypophallus straight-point shape (Char 11, state 1), a narrow pseudophallus (Char 13, state 2), the pseudophallus extending beyond the right epiphallus (Char 17, state 1), and the curvature of the titillator (Char 20, state 2). When looking at the two major clades of *Tenodera*, the (*T. costalis* (*T. superstitiosa* + *T. bokiana*)) clade is supported by traits derived from the

acutolobus, the main one being the angle of the acutolobus in relation to the left epiphallus (Char 0, state 1). The second major clade (*T. australasiae* (*T. sp. nov.* (*T. aridifolia* + *T. sinensis*))) is supported only by the bend of the pseudophallus under DELTRAN (Char 14, state 1). The (*T. sp. nov.* (*T. aridifolia* + *T. sinensis*)) clade has several synapomorphies including: the angle of the acutolobus in relation to the left epiphallus (Char 0, state 1), an up-curved hypophallus point (Char 9, state 1), the same overall shape of the pseudophallus (Char 13, state 1), and the location of the bend in the pseudophallus (Char 15, state 1). Further the (*T. aridifolia* + *T. sinensis*) clade differed in the left margin of the pseudophallus stalk (Char 23, state 1). Looking at the three species we found that *T. aridifolia* had character states differentiating it from the other two, namely: the hypophallus orientation (Char 7, state 0) and the size of the small lobe of the right epiphallus (Char 18, state 2). *T. sinensis* has an up-curved point on the acutolobus (Char 3, state 1).



**Fig. 3.** Comparison between the morphology-only tree and the combined-data tree. Numbers at the nodes indicate the number of morphological synapomorphies from the genitalia that support that node.

## Discussion

### Phylogeny of *Tenodera*

Our study presents the first explicit phylogenetic hypothesis of *Tenodera*. A monophyletic *Tenodera* is strongly supported. In light of the present phylogeny, it appears that the traditionally used character systems (Giglio-Tos 1927; Kaltenbach 1996), while useful for diagnosing species, are inadequate for phylogenetic inference among species. For instance, the main character that had been used to distinguish *T. aridifolia* from *T. sinensis* was overall body size, where the former is known to be smaller than the latter, but small size appears to have evolved at least three times in the genus (*T. australasiae*; *T. aridifolia* and *T. sp. nov.*; *T. costalis*, *T. superstitiosa* and *T. bokiana*). With the genital characters used in this study, however, it is possible to confidently distinguish between these two species (Fig. 1). Based on discrete genital characters such as the shape of the acutolobus and the orientation of the hypophallus, we treat *T. sinensis* as a distinct species from *T. aridifolia*. Furthermore, we have identified what appears to be a novel lineage that was initially identified as unique but similar to *T. aridifolia*. It is found only in India, distinct from the Indo-Malaysian distribution of *T. aridifolia*. Molecular data consistently separate this lineage from *T. aridifolia* (Figs 1, 2) and the shape of the hypophallus is also unique (Fig. 4G). Although this lineage is likely to represent a new species, we are hesitant to describe it as such because our finding is based on a single specimen. More specimens will be necessary to document intraspecific variation to firmly establish the identity of this species. Our phylogeny recovers *T. superstitiosa* and *T. bokiana* as two genetically well defined separate species that are sister to each other (Figs 1 and 2). Other than the colour of the discoidal spines on the fore legs, we have identified the orientation and shape of the acutolobus in male genitalia to be discrete characters that separate the two species. The disjunct distribution of these two species, *T. superstitiosa* being an African species and *T. bokiana* being an Indo-Pacific

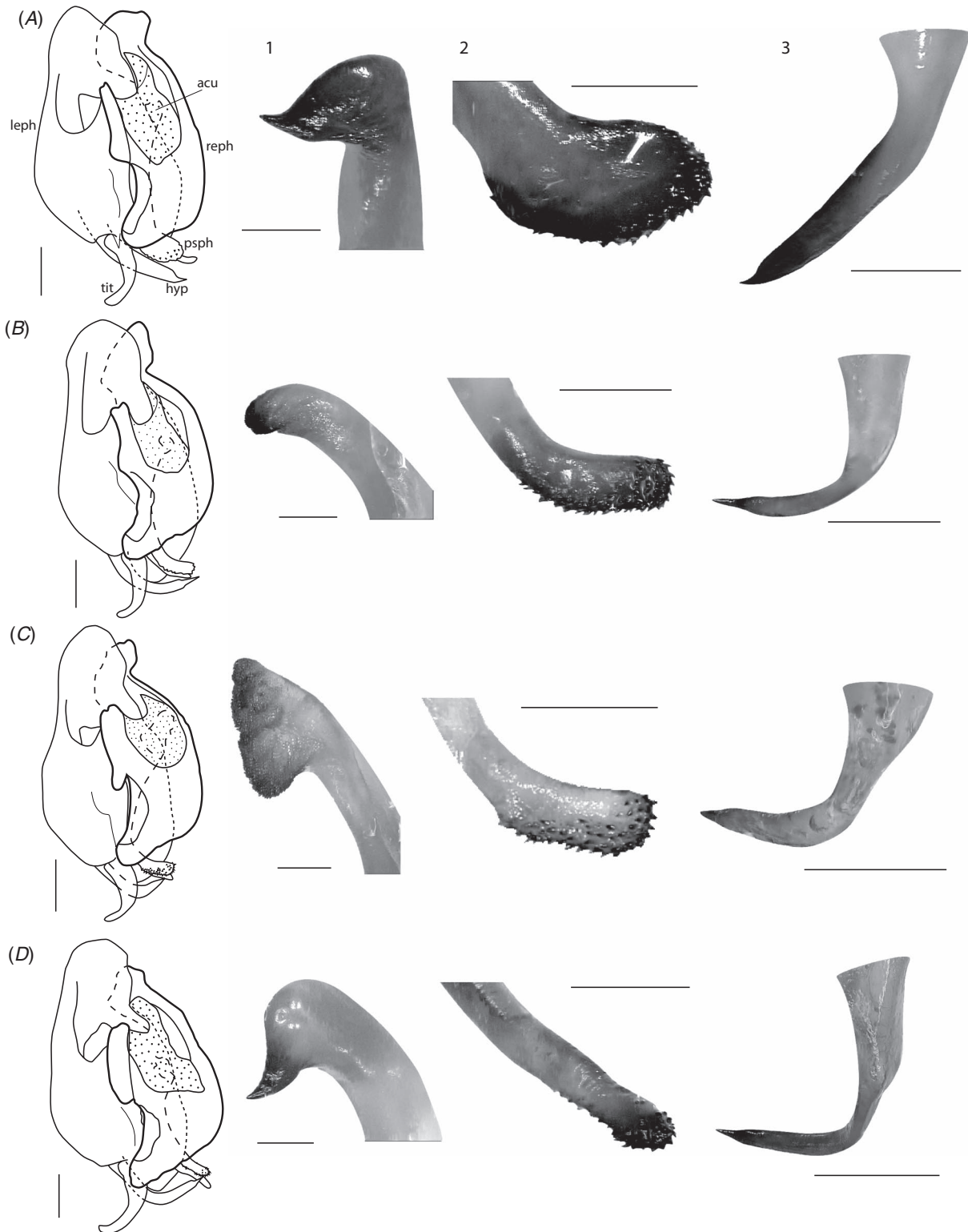
species, is also suggestive of the species status of these two species.

The relationships of *T. australasiae* appear to be unstable. A close examination of genital structures suggests that it is morphologically similar to *T. superstitiosa* and *T. bokiana* because all three species have a blade-shaped acutolobus as well as a similarly shaped hypophallus (Fig. 4B, C, F). The molecular characters, however, did not group these three species together. It is possible that *T. australasiae* represents a divergent lineage with convergent forms of genital structure (Figs 2, 5) and more support could be found with the addition of more molecular data. Since our analysis represents about half of the species of *Tenodera*, this relationship might be resolved with the addition of other tenoderan species from the Indo-Pacific region.

### Taxonomic and phylogenetic utility of male genitalia in *Tenodera*

By mapping genital characters on the MP topology based on all of the data (Fig. 5), we find several general trends in the individual genital components in *Tenodera* compared with the outgroups. The shape of the titillator is either hooked or straight and flat in the outgroups and it becomes curved dorsally in *Tenodera* (Fig. 5). The smooth-surfaced acutolobus is a character that is shared between *Tenodera* and the outgroups although the shape of this structure does evolve differently in *Tenodera* species. The pseudophallus is shorter than the margin of the right epiphallus in the outgroups, but it extends beyond the apex of the right epiphallus in *Tenodera*. Also, *Tenodera* species display a unique bend in the pseudophallus that is absent in outgroup taxa (Fig. 5). Several outgroups have extra extensions protruding off of the hypophallus, but none of the *Tenodera* species have such extensions beyond the main structure of the hypophallus.

Within *Tenodera*, several interesting evolutionary progressions are found in different genital components (Fig. 4). The acutolobus is located on the right of the left epiphallus and situated on the end of a stalk that connects it with the pseudophallus. It can be viewed only after a full dissection of the genitalia separating the left and right epiphalli. The size of the acutolobus was mostly consistent throughout the species. Several species, including *T. aridifolia* (Fig. 4A), *T. sinensis* (Fig. 4E), *T. sp. nov.* (Fig. 4G) and *T. costalis* (Fig. 4D), display a smooth-surfaced acutolobus (Fig. 4: structure 1) that ends distally in a spine, similar to the outgroups, although the shape of the spine differs in the outgroups. Of these four species, *T. costalis* (Fig. 4D) belongs to a separate clade and its acutolobus differs greatly in its orientation, being at 180° to the left epiphallus and at 45° to the pseudophallus stalk, while maintaining a very similar shape to the other species in the *T. aridifolia* clade (Fig. 4A, E, G). The shape of acutolobus (Fig. 4: structure 1) is different in *T. superstitiosa* (Fig. 4F), *T. bokiana* (Fig. 4C) and *T. australasiae* (Fig. 4B), in that it is a shape of a blade, without a spike, and very rough in texture. When optimised onto the phylogeny, this blade-shaped acutolobus appears to have evolved at least twice, once in the common ancestor of *T. superstitiosa* and *T. bokiana*, and once in *T. australasiae*



**Fig. 4.** Overall view of non-dissected male genitalia from seven *Tenodera* exemplars: (A) *T. aridifolia*, (B) *T. australasiae*, (C) *T. bokiana*, (D) *T. costalis*, (E) *T. sinensis*, (F) *T. superstitiosa* and (G) *T. sp. nov.* The parts labelled in species *A* (and apply to species *A*–*G*) are the left epiphallus (leph), right epiphallus (reph), titillator (tit), acutolobus (acu), pseudophallus (psph), and the hypophallus (hyp). The acutolobus is not clearly visible owing to its position under a membranous section of the right epiphallus, which requires further dissection of the genitalia. The scale bar equals 1 mm. To the right of each illustration are images of three structures investigated in this study: Structure 1 = acutolobus (scale bar = 0.25 mm); Structure 2 = pseudophallus (scale bar = 0.5 mm); Structure 3 = hypophallus (scale bar = 1 mm).



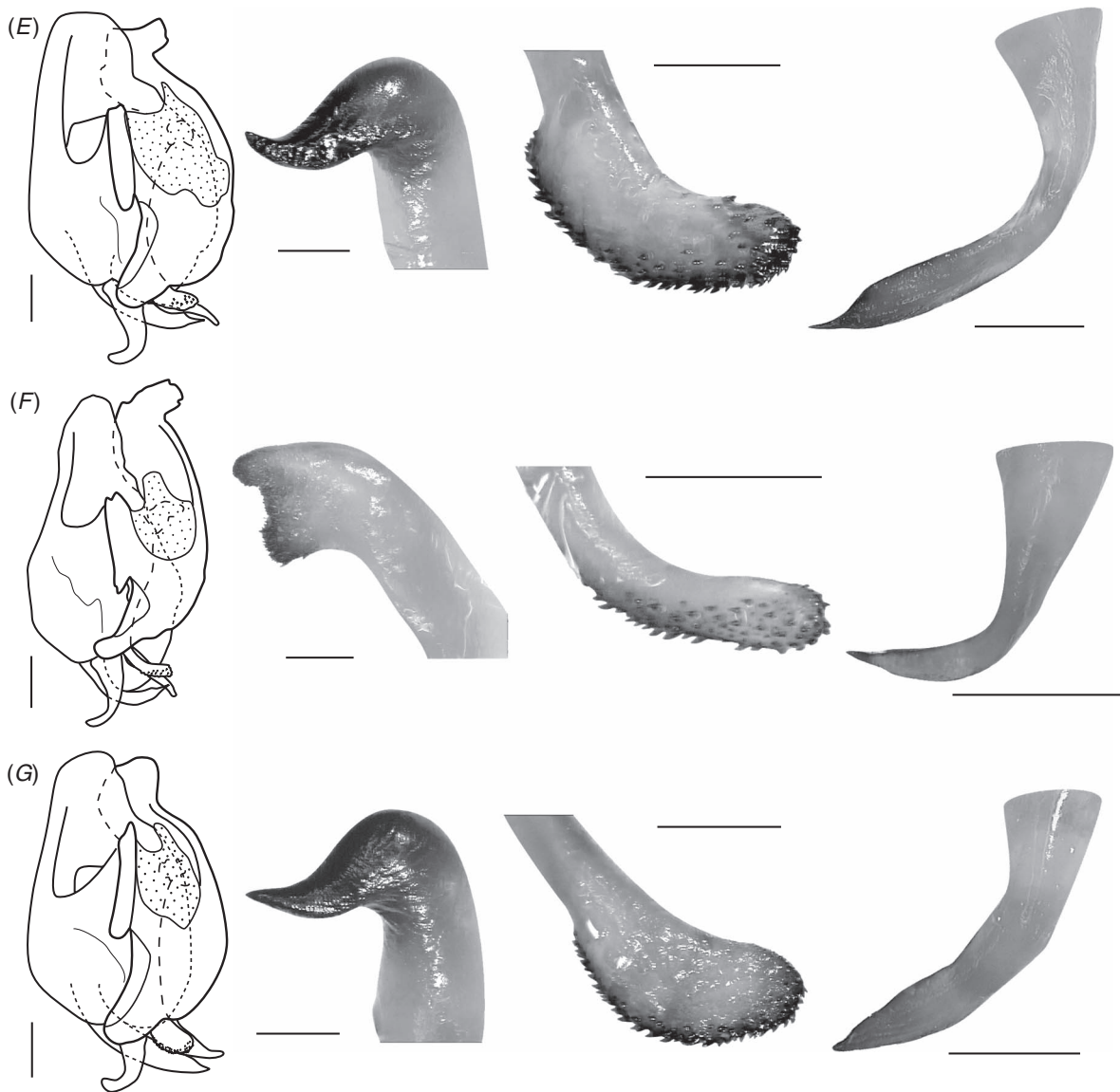
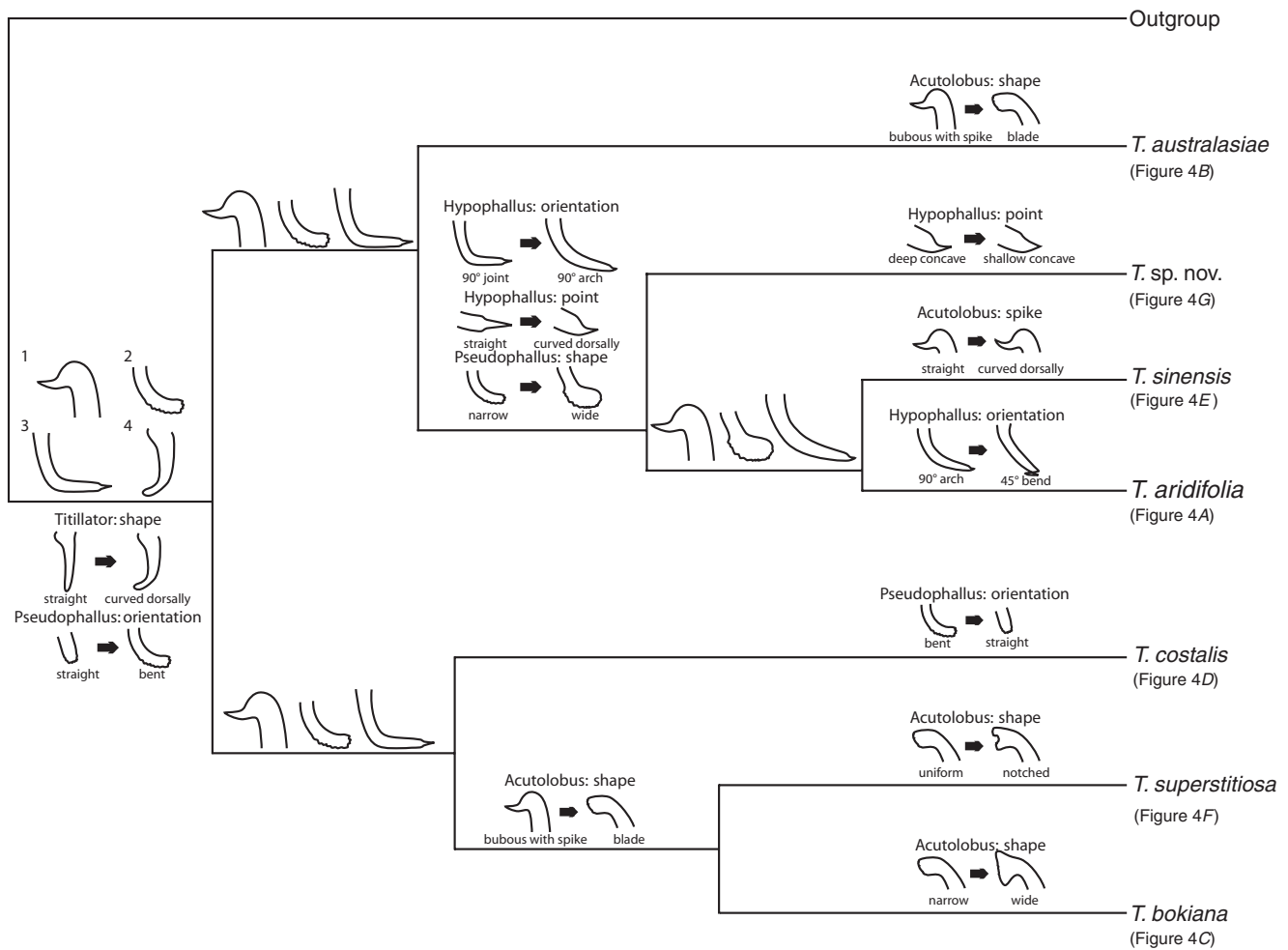


Fig. 4. (continued)

(Fig. 5). The pseudophallus is located at the base of the pseudophallus stalk on the right side of the left epiphallus. *Tenodera aridifolia*, *T. sinensis* and *T. sp. nov.* have a pseudophallus (Fig. 4: structure 2) that is much wider posterior to the bend than the other species (Fig. 5). This bend also occurs near to the middle of the basal spines. *Tenodera aridifolia* (Fig. 4A) and *T. sinensis* (Fig. 4E) display a more acute angle of a bend compared a more obtuse bend in *T. sp. nov.* (Fig. 4G). The remaining four species display a narrow pseudophallus (Fig. 4B–D, F; Fig. 5). These all bend before the spines except for the pseudophallus of *T. costalis* (Fig. 4D). Its pseudophallus does not bend and is more similar to the outgroups in that respect, though it does extend beyond the right epiphallus, similar to the other *Tenodera* species. The hypophallus (Fig. 4: structure 3) is a comparatively large and sword-shaped structure that originates from the posterior of the left epiphallus and extends to the right

below the pseudophallus. *Tenodera aridifolia* (Fig. 4A), *T. sinensis* (Fig. 4E) and *T. sp. nov.* (Fig. 4G) each have a much wider hypophallus with a uniquely pointed terminus compared with those of the other species. The terminal end of the hypophallus in these three species has distinct concavity dorsally and the overall structure is loosely bent. The remaining four species (Fig. 4B–D, F) display a concavity on both dorsal and ventral sides of the terminal end of the structure and a 90° bend midway down the hypophallus.

The character optimisation suggests that certain features of male genitalia are phylogenetically conserved while other features are homoplasious. For example, *T. aridifolia* (Fig. 4A), *T. sinensis* (Fig. 4E) and *T. sp. nov.* (Fig. 4G) share several characters, while species-specific differences do exist. *Tenodera superstitiosa* (Fig. 4F) and *T. bokiana* (Fig. 4F) share numerous synapomorphies despite the fact that the former is



**Fig. 5.** Tree showing the reconstruction of transitions of genital structures across the phylogeny focusing on the four main structures: (1) acutolobus, (2) pseudophallus, (3) hypophallus and (4) titillator. Ancestral states of the certain transitional structures are depicted on ancestral node and were optimized under either ACCTRAN or DELTRAN. State transitions are illustrated on branches. For each node uniting a species, we included the structure that serves as the diagnostic genital feature for that species.

distributed in Africa and the latter in Australasia (Table 1). Two species in particular, *T. costalis* (Fig. 4D) and *T. australasiae* (Fig. 4B), display a unique combination of traits (Appendices 1 and 2). For example, *T. costalis* is placed near the *T. superstiosa* clade in the phylogeny, but has an acutolobus similar to the *T. aridifolia* clade (Figs 1; 4A, E, G). The phylogenetic placement of *T. australasiae* is close to the *T. aridifolia* clade, whereas its genitalia are morphologically similar to the *T. superstiosa* clade (Figs 1; 4C, F).

#### Evolution of male genitalia in Tenodera

Our finding suggests that the phylogenetic signal from male genitalia alone may be insufficient to resolve relationships among individual species of *Tenodera*, although the monophyly of the genus was strongly supported by several genital characters (Fig. 3). This is an unexpected result because male genital characters are often very informative in resolving phylogenetic relationships at various levels (Song and

Bucheli 2009). Our genital character matrix contains several characters that are useful for grouping species within the genus, with a relatively low level of overall homoplasy (CI: 0.69) and a rather high level of overall synapomorphy (RI: 0.70), but it also contains several characters that conflict with each other. Eight characters included in the matrix have a *CI* lower than 0.5 (Appendix 1), which implies that about one-third of the genital characters is highly homoplasious. In contrast, molecular data alone were able to resolve the relationships unambiguously (Fig. 2). It is certainly possible that the difference in resolution between morphology and molecules may reflect the difference in size of the datasets (24 genital characters versus 2270 nucleotide characters). However, it is also possible to speculate that the rate of evolution in male genitalia may actually be higher than molecular divergence among species. For example, sequence divergence between *T. aridifolia* and *T. sinensis* is low, which can be inferred from a short branch length between two species clades (Fig. 2), but each of them has a unique and distinct set of genital characters (Fig. 4A, E), which implies that the rate of

morphological divergence may be rapid. Theoretical studies suggest that traits under sexual selection, such as male genitalia, evolve very rapidly (Eberhard 1985; Hosken and Stockley 2004), and in some cases there is little correspondence between phenotypic similarity in male genitalia and phylogenetic similarity across species (Arnqvist and Rowe 2002). Added selective pressure from sexual conflict in cannibalistic mating systems (Miller 2007) may accelerate the rate of genital evolution in mantises, and our study may be an example of such rapid genital evolution.

In studying the evolution of male genitalia, it is important to understand the function of each genital structure. Although it is difficult to identify the exact function of each genital component without explicit studies, we can speculate on the putative function based on the shape and sculpting pattern (Fig. 4). The left epiphallus consists of three horned processes that are likely to serve an important function during copulation (Tuxen 1956). The acutolobus is likely to serve as a hook during copulation or a sensory structure because it is covered with sensillae in some species. The pseudophallus is covered with small spines that are curved backward, which could damage the membranous parts of female genitalia during copulation. The titillator often has an apex that is curved outwards, which may be used for physically connecting with the female during copulation. The hypophallus serves as the opening of the ejaculatory duct, and therefore can be considered an intromittent organ.

Classic theory of sexual selection by cryptic female choice predicts a rapid divergence in male genital shape by a Fisherian runaway selection (Fisher 1930; Eberhard 1985). In *Tenodera*, the titillator, the acutolobus and the hypophallus may be possible candidates as the internal courtship devices that would be affected by the female choice. We find that the titillator is relatively stable across species, whereas the acutolobus shape is wildly divergent. The hypophallus is variable across species, but the degree of variation is low compared with that in the acutolobus. Female mantises can mate multiple times (Maxwell 1999) and there appears to be no obvious sperm precedence. Such life history traits suggest that cryptic female choice might drive the evolution of male genitalia in mantises.

Male mantises face a very high cost of mating owing to the sexually cannibalistic behaviour of females. Hurd *et al.* (1994) suggested that females continue to attract and cannibalise males beyond their need for sperm, to alleviate food limitation during oogenesis, and there appears to be no direct benefit for males to engage in sexual cannibalism (Lelito and Brown 2006). Although genital mutilation reported from spiders (Miller 2007) does not appear to happen in mantises because there is no genital structure that can break off during copulation, the pseudophallus does contain numerous spines that could damage female genital tracts during copulation. Genital damage has been observed in the bean weevil (*Callosobruchus maculata*) where male genitalia have sclerotised spines that wound female counterparts (Crudginton and Siva-Jothy 2000). This type of genital damage can increase the fitness of the male by reducing the risk of sperm competition and by increasing the immediate oviposition rates. Armored genitalia may evolve rapidly in response to sexual cannibalism, and male genitalia might experience a strong selective pressure to evolve a morphology

that is effective in damaging females in order to shift the fitness equation in favour of adaptive male sacrifice (Miller 2007). Therefore, two different selective pressures, sexual selection by cryptic female choice and male complicity resulting from sexual cannibalism, may ultimately drive the rapid evolution of male genitalia in *Tenodera*. The mating behaviour of *Tenodera* thus presents an exciting research system and explicit mating experiments with respect to male fitness might shed light on the evolution of male genitalia.

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**Appendix 1. Male genitalia character descriptions and states**

The characters listed here are in reference to several structures of mantis male genitalia pictured in Fig. 4 (see also Fig. 5)

- (0) *The angle of the acutolobus in relation to the left epiphallus*: (0) 90° angle; (1) 45° angle; (2) 180° angle. (CI=0.50, RI=0.60)  
 (1) *The angle of the acutolobus in relation to the stalk*: (0) 90° angle; (1) 45° angle. (CI=0.33, RI=0)  
 (2) *The overall shape of the acutolobus*: (0) apically bulbous with blunt point; (1) apically bulbous with spine; (2) blade; (3) fan; (4) spine. (CI=0.80, RI=0.75)  
 (3) *Of those taxa with state 1 of char 2, the acutolobus spine orientation*: (0) straight; (1) curved dorsally. (CI=0.50, RI=0)  
 (4) *Of those taxa with state 2 of char 2, the acutolobus blade notch*: (0) absent; (1) present. (Uninformative)  
 (5) *Of those taxa with state 2 of char 2, the acutolobus blade width compared with width of stalk*: (0) narrow; (1) wide. (Uninformative)  
 (6) *The nature of the surface of the acutolobus*: (0) smooth; (1) rough, small spines present. (CI=0.33, RI=0.50)  
 (7) *The hypophallus orientation (relative to the base of the left epiphallus)*: (0) 45° bend; (1) 90° arch; (2) Straight with a 90°/right angle joint. (CI=0.66, RI=0.66)  
 (8) *The shape of the distal end of the hypophallus*: (0) point; (1) round end; (2) straight with a notch. (CI=1, RI=1)  
 (9) *Of those taxa with state 0 of char 8, the hypophallus point shape*: (0) straight point; (1) curved dorsally. (CI=1, RI=1)  
 (10) *Of those taxa with state 1 of char 9, the hypophallus point concavity*: (0) minimal; (1) deep. (Uninformative)  
 (11) *Of those taxa with state 0 of char 9, the hypophallus point shape*: (0) gradual point; (1) point preceded by indents on base and apex of hypophallus. (CI=1, RI=1)  
 (12) *The hypophallus width*: (0) narrows at distal portion; (1) widens at distal portion. (CI=0.50, RI=0)  
 (13) *The overall shape of the pseudophallus*: (0) flat distal end; (1) wide and rounded; (2) narrow; (3) bulbous. (CI=1, RI=1)  
 (14) *The orientation of the pseudophallus*: (0) straight; (1) bent. (CI=0.50, RI=0.80)  
 (15) *Of those taxa with state 1 of char 14, origin of pseudophallus bend*: (0) before spines; (1) near middle of basal spines. (CI=1, RI=1)  
 (16) *Of those taxa with state 1 of char 15, the pseudophallus bend angle*: (0) obtuse angle; (1) right angle. (Uninformative)  
 (17) *Extension of the pseudophallus beyond the apex of the right epiphallus*: (0) no; (1) yes. (CI=0.50, RI=0.50)  
 (18) *The size of the small lobe on the left side of the right epiphallus*: (0) absent; (1) pronounced; (2) small. (CI=0.50, RI=0.50)  
 (19) *Extra extensions of the hypophallus*: (0) absent; (1) spike; (2) ridge. (CI=1, RI=1)  
 (20) *The shape of the titillator*: (0) hooked; (1) straight; (2) curved dorsally. (CI=1, RI=1)  
 (21) *The presence of small spines on the left side of the right epiphallus*: (0) absent; (1) present. (CI=1, RI=1)  
 (22) *The hypophallus origin*: (0) off base of left epiphallus; (1) out of posterior of left epiphallus. (CI=1, RI=1)  
 (23) *The pseudophallus stalk left margin*: (0) straight; (1) depression between two ridges; (2) one ridge; (3) indent near acutolobus. (CI=0.60, RI=0.60)

**Appendix 2. Male genitalia character matrix**

Taxa	Characters		
	0000000000	1111111111	2222
	0123456789	0123456789	0123
<i>Ta. tamolana</i>	000---1100	-0000--001	0103
<i>He. schultzei</i>	000---1100	-0000--001	0103
<i>Po. aeruginosa</i>	113---012-	--030--000	0013
<i>Pl. guineensis</i>	004---011-	--030--102	1010
<i>Pr. ornatipennis</i>	004---011-	--030--102	1010
<i>T. sp. nov.</i>	1010--0101	0-11110110	2010
<i>T. sinensis</i>	1011--0101	1-11111110	2011
<i>T. aridifolia</i>	1010--0001	1-01111120	2011
<i>T. australasiae</i>	002-001100	-10210-100	2012
<i>T. costalis</i>	2111--0200	-1020--120	2010
<i>T. superstitiosa</i>	212-111200	-10210-110	2012
<i>T. bokiana</i>	102-011200	-10210-110	2012