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Re-evaluation of taxonomic utility of male phallic complex in higher-level classification of Acridomorpha (Orthoptera: Caelifera)

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Abstract
The current higher classification of the orthopteran superfamily group Acridomorpha is largely based on interpretation of male phallic structures. Internal male genitalia have been considered as an excellent taxonomic character because of a widespread belief that they are less subject to selective pressures from environment, and thus more stable than external characters. Furthermore, based on a notion that evolution proceeds from simple to complex, early taxonomists who shaped the higher classification of Acridomorpha considered those groups with less differentiated and membranous phallic structures as primitive and used this notion to deduce a phylogeny of Acridomorpha. In this study, we test these ideas based on a cladistic analysis of male phallic structures and a character optimization analysis to assess the level of homoplasy and synapomorphy for those phallic characters that have been traditionally used for the Acridomorpha systematics. We also perform an independent test of the phylogenetic utility of male phallic structures based on a molecular phylogeny. We show that while some phallic structures have strong phylogenetic signal, many traditionally used characters are highly homoplasious. However, even those homoplasious characters are often informative in inferring relationships. Finally, we argue that the notion that evolution proceeds in increasing complexity is largely unfounded and difficult to quantify in the higher-level classification of Acridomorpha.

Keywords
Male genitalia; homoplasy; complexity

Introduction

Male genitalia are arguably among the most important and versatile morphological characters in insect taxonomy (Tuxen 1970; Song & Bucheli 2010). Male genitalia possess many traits that are unique to species, especially among closely related species, and their utility in species diagnosis has been thoroughly proven in many groups (Eberhard 1985; Hosken & Stockley 2004). They also provide excellent phylogenetic

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signal when compared across divergent lineages and higher-level classifications of several insect orders are largely based on male genitalia (Sharp & Muir 1912; Eyer 1924; Peck 1937; Michener 1944; Zumpt & Heinz 1950; Snodgrass 1957; Roth 1970). In general, insect male genitalia can be considered a composite character that consists of several functionally independent units that might evolve at different rates (Huber 2004; Song & Wenzel 2008; Song 2009; Song & Bucheli 2010).

Male phallic structures have played a particularly important role in inferring higher-level classification in the superfamily group Acridomorpha within Caelifera, the smaller of the two suborders within Orthoptera. The monophyletic Acridomorpha consists of six superfamilies, Acridoidea, Eumastacoidea, Pneumoroidea, Pyrgomorphoidea, Tanaoceroidea and Trigonopterygoidea, which are grasshopper-like in morphology (Dirsh 1975; Flook & Rowell 1997; Song 2010). Twenty-four extant families including more than 9500 described species are recognized in Acridomorpha and the current classification is largely based on interpretation of male phallic structures (Roberts 1941; Dirsh 1956; Barnum 1959; Kevan et al. 1969; Descamps 1973; Amédégnato 1976; Jago 1989; Eades 2000; Song 2010). A succinct review of the use of male phallic structures in grasshopper systematics can be found in Song (2010).

A typical male phallic complex of Acridomorpha consists of three concentric layers that make up endophallus, ectophallus and epiphallus (Amédégnato 1976) and the whole organ is thought to be derived from ectoderm (Dirsh 1956; Kevan et al. 1969). The endophallus is often a heavily sclerotized structure consisting of a pair of valves and the endophallic sac. The basal valves of the penis function as apodemes for muscles that are used for pumping spermatophore during copulation, and the apical valves represent an actual intromittent organ (Eades 2000; Song 2004). The ectophallus encapsulates the endophallus and consists of cingulum and differentiated ectophallic sclerites in some species (Amédégnato 1976). The ectophallus appears to protect the endophallus and to provide muscle attachment sites, and based on an observation that the apical portion is often covered with sensillae, it might be involved in copulatory courtship (Song & Wenzel 2008). The epiphallus is a strongly sclerotized structure, located dorsally of the ectophallus. In many grasshopper species, there is a pair of hook-like structures known as lophi, which serve as a grasping organ that hooks on to the base of the female subgenital plate during copulation (Randell 1963). There is an enormous amount of variation in each component of male phallic structures across different lineages of Acridomorpha, which is shown to be extremely useful not only for species diagnosis (Hubbell 1932), but also for higher-level classification. A generalized figure of a typical grasshopper phallic complex is shown in Figure 1.

Roberts (1941) was the first taxonomist to use male genitalia for the higher-classification of Acridomorpha, who argued that these characters provide excellent phylogenetic signal, unlike external morphological traits that are easily influenced by environment. Subsequent taxonomists, including Dirsh (1956), Kevan et al. (1969), Amédégnato (1976) and Eades (2000), all found a similar pattern, suggesting that male genitalia should be more stable and reflect correct phylogenetic relationships better than external morphology because phallic structures are internal and thus free from

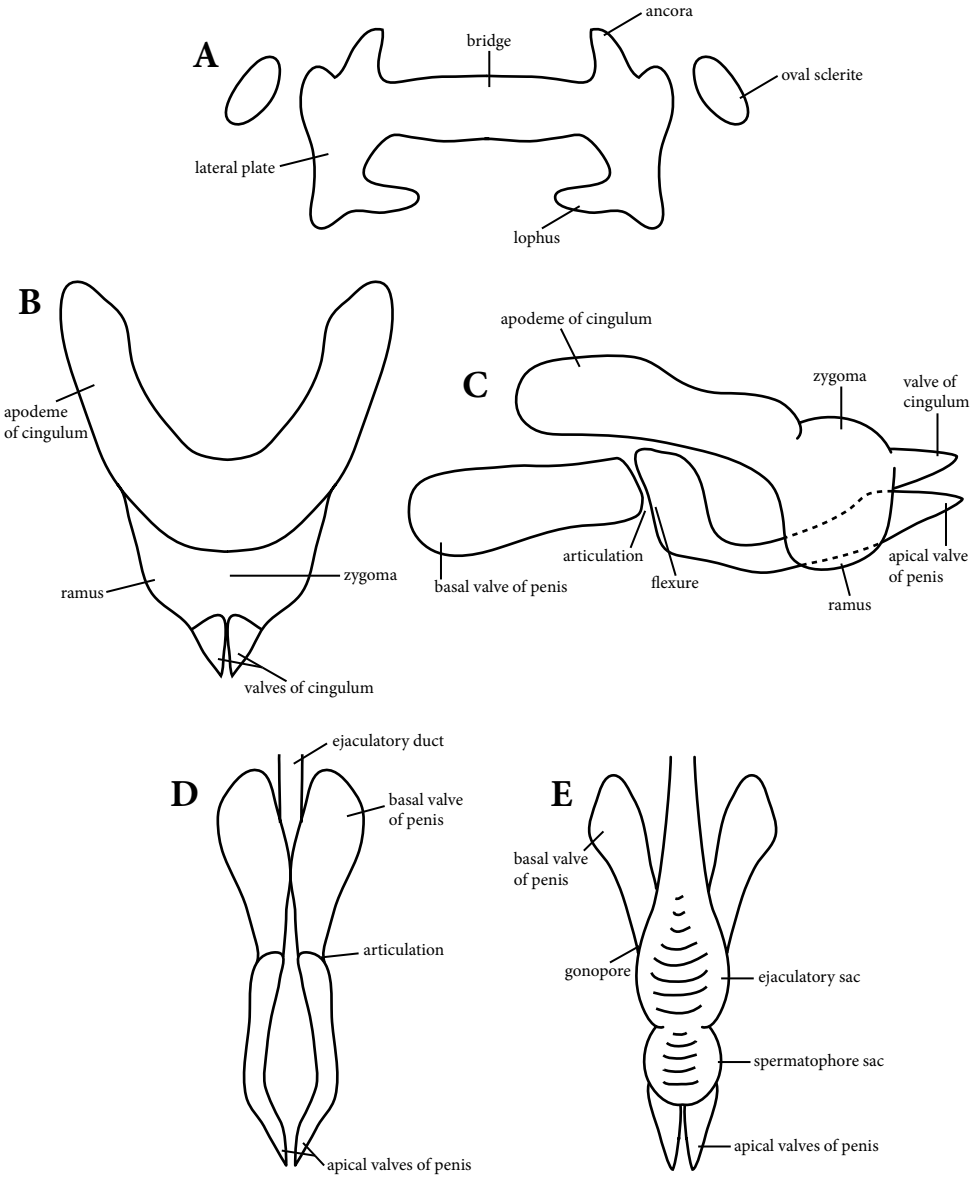

Fig. 1. A generalized morphology of the male phallic complex of Acridomorpha. (A) Epiphallus; (B) Dorsal view of cingulum (ectophallus); (C) Lateral view of cingulum and endophallus; (D) Dorsal view of endophallus; (E) Ventral view of endophallus.

selective pressures from environmental variations. However, a large body of theories on genital evolution posits that animal male genitalia evolve rapidly and divergently, possibly due to sexual selection (Eberhard 1985, 2010; Alexander et al. 1997; Arnqvist 1998; Hosken & Stockley 2004). This suggests that the notion of male genitalia being "stable" may not be an accurate one.

When developing their phylogenetic hypotheses, the early grasshopper taxonomists mostly relied on a notion that evolution proceeds from simple to complex, rather than based on a character-based cladistic analysis (Dirsh 1956; Amédégnato 1976; Eades 2000). For example, some lineages, such as Eumastacoidea, have a single, rod-shaped endophallus and no discernable ectophallus, which they considered simple and primitive. On the other hand, male genitalia of Acrididae were considered to be complex and advanced because of their well-differentiated endophallus and cingulum (Dirsh 1956). Across Acridomorpha, lineages with fewer genital parts and less differentiations have been considered basal, and this idea is reflected in the current classification scheme of Acridomorpha (Dirsh 1975; Kevan 1982). Furthermore, because different authors subscribed to different interpretations of homology of phallic characters across different families (Table 1), their classification schemes often contradicted with each other (Song 2010). Importantly, these ideas have never been formally tested in an explicit phylogenetic context.

In this study, we re-evaluate the taxonomic utility of the male phallic complex in the higher-level classification of Acridomorpha in a phylogenetic framework. Based on a cladistic analysis of phallic characters as well as a recently available molecular phylogeny of Acridomorpha, we will test the following hypotheses: (i) How much phylogenetic signal do genital structures possess and are they free of homoplasy?; and (ii) Do male genitalia evolve from simple to complex in Acridomorpha?

Materials and Methods

Molecular phylogeny

The senior author recently completed a phylogenetic analysis of the grasshopper superfamily Acridoidea based on complete mitochondrial genome sequence data (Leavitt et al. 2013). Based on 15 338 aligned nucleotides and 34 terminals representing all eight superfamilies within Caelifera, this study represented the most rigorous analysis of the superfamily to date. Although the focus of the phylogenetic analysis was to test the monophyly of Acridoidea, the study included major representatives of Acridomorpha and resulted in well-resolved relationships among major families and superfamilies with strong supports. From the best topology presented in Leavitt et al. (2013), we collapsed several nodes to families to obtain a family-level phylogeny of Acridomorpha. This final topology was used as an independent test for studying the evolution of the male phallic complex in Acridomorpha.

Characterization of the male phallic complex

We reviewed descriptions of male phallic structures as interpreted by Amédégnato (1976), Dirsh (1956), Eades (2000) and Roberts (1941) (Table 1), and compiled a list of characters that have been shown to be informative for the higher-level classification within Acridomorpha. Based on the data extracted from the literature, we identified a total of 26 characters from epiphallus, ectophallus and endophallus that could

Table 1. Comparison of terminologies across four major studies on the male phallic complex of Acridomorpha.

	Roberts (1941)	Dirsh (1956)	Amédégnato (1976)	Eades (2000)
Epiphallus	ancora of epiphallus	ancorae	–	ancora of epiphallus
	bridge of epiphallus	bridge	pont	bridge of epiphallus
	lophus	lophi	–	lophus of epiphallus
	lateral sclerite of epiphallus	oval sclerites	–	oval sclerite
	lateral plate of epiphallus	lateral plates	–	–
	–	posterior projections	–	–
	–	median slit	–	–
	–	–	membrane épiphallique	epiphallic infold
	–	anterior projections	–	–
	–	median projection	–	–
Ectophallus	pallium	pallium	pallium	pallium
	sheath of aedeagus	sheath of penis	gaine	sheath
	ramus of cingulum	rami of cingulum	rami du cingulum	ramus of cingulum
	zygoma of cingulum	zygoma	zygoma	zygoma of cingulum
	cingulum	cingulum	cingulum	cingulum
	apodeme of cingulum	apodemes	apodémes du cingulum	apodeme of cingulum
	dorsal valves of aedeagus	valves of cingulum	valves supérieurs de l'édéage	dorsal aedeagal valve
	dorsal valves of aedeagus of penis	dorsal appendices	–	–
	arch of dorsal valves	arch of cingulum	arche du cingulum	arch
	basal fold	basal fold	pli basal	basal fold
	–	ectophallic membrane	membrane ectophallique	central membrane
	–	–	supra rami	supraramus of cingulum
	ectophallic sclerite	ectophallic sclerite	sclérites supérieurs de l'édéage	dorsal aedeagal sclerite
	–	ventral fold	–	–
	ventral infold	ventral infold	–	ventral infold
	ventral lobe	ventral lobe	–	ventral lobe
	–	ventral fold	–	–
Endophallus	ejaculatory sac	ejaculatory sac	sac éjaculateur	ejaculatory sac
	spermatophore sac	spermatophore sac	sac spermatophore	spermatophore sac
	flexure	flexure	flexure	–
	ejaculatory duct	ejaculatory duct	–	ejaculatory duct
	gonopore	gonopore	gonopore	–
	gonopore process	gonopore process	processus du gonopore	–
	aedeagus	penis	–	–

(*Continued*)

Table 1. (*Cont.*)

	Roberts (1941)	Dirsh (1956)	Amédégnato (1976)	Eades (2000)
	phallotreme	phallotreme	–	phallotreme
	ventral valve of aedeagus	apical valves of penis	valves inférieurs de l'édéage	ventral aedeagal valve
	–	ventral appendices of penis	–	–
	articulation	articulation	–	–
	endophallic plate	basal valves of penis	apodémes endophalliques	endophallic apodeme
	cleft	cleft	–	ventral phallotreme cleft
	–	–	–	dorsal phallotreme cleft
	–	endophallic membrane	–	phallotreme membrane
	endophallic sclerites	endophallic sclerites	sclérites inférieurs de l'édéage	ventral aedeagal sclerite
	–	valves of the ejaculatory duct	–	–

adequately characterize each family. First we extracted information about intra-familiar variation from literature data by examining character descriptions and accompanying figures of phallic structures. However, the literature data were often incomplete and vague, and some figures were too stylized to be interpreted with confidence. Therefore, we confirmed morphological variations by examining male phallic structures dissected from museum specimens of representative families (except Pyrgacrididae, which was not available). The museum specimens were first relaxed in a relaxing chamber for 48 h and male genitalia were dissected by slitting open the membrane between epiproct and subgenital plate. The phallic complex was extruded by inserting a tip of forceps under the ventral portion of the structure and by gently pulling it. Dissected phallic structures were first placed in a weak KOH solution for three to four hours to dissolve muscle. Cleared structures were stored in genital vials with glycerin. High-resolution photographs of the male phallic structures were taken with a BK Plus Imaging System (Visionary Digital). We included the following acridomorph families, representing six superfamilies for the analysis: Eumastacoidea (Eumastacidae), Tanaoceroidea (Tanaoceridae), Trigonopterygoidea (Trigonopterygidae), Pneumoroidea (Pneumoridae), Pyrgomorphoidea (Pyrgomorphidae) and Acridoidea (Charilaidae, Pamphagidae, Pyrgacrididae, Lentulidae, Lithidiidae, Tristiridae, Ommexechidae, Romaleidae and Acrididae). A detailed list of dissected specimens is shown in Table 2.

Phylogenetic analysis and character optimization

From the literature data and the museum specimens, we identified a total of 26 characters with 56 states across the 14 acridomorph families, which were converted into a

Table 2. Taxonomic information on specimens dissected in this study.

Superfamily	Family	Species	Collecting locality	Museum
Eumastacoidea	Eumastacidae	*Paramastax nigra* (Scudder, 1875)	Peru	ANSP
Tanaoceroidea	Tanaoceridae	*Tanaocerus koebelei* Bruner, 1906	USA	ANSP
Trigonopterygoidea	Trigonopterygidae	*Systella philippensis* (Walker, 1870)	Philippines	ANSP
Pneumoroidea	Pneumoridae	*Physemacris variolosa* (Linnaeus, 1758)	South Africa	BMNH
Pyrgomorphoidea	Pyrgomorphidae	*Atractomorpha aberrans* Karsch, 1888	Congo	ANSP
Acridoidea	Charilaidae	*Hemicharilaus monomorphus* (Uvarov, 1929)	Namibia	ANSP
Acridoidea	Pamphagidae	*Prionotropis hystrix hystrix* (Germar, 1817)	Croatia	ANSP
Acridoidea	Lentulidae	*Lentula* sp.	South Africa	ANSP
Acridoidea	Lithidiidae	*Lithidiopsis carinatus* (Dirsh, 1956)	South Africa	BMNH
Acridoidea	Tristiridae	*Moluchacris cinerascens* (Philippi, 1863)	Chile	ANSP
Acridoidea	Ommexechidae	*Ommexecha virens* (Serville, 1831)	Brazil	ANSP
Acridoidea	Romaleidae	*Xyleus laevipes* (Stål, 1878)	Argentina	ANSP
Acridoidea	Acrididae	*Guaranacris specularis* (Bruner, 1906)	Paraguay	ANSP

ANSP, Academy of Natural Sciences, Philadelphia, PA; BMNH, The Natural History Museum, London.

matrix for a cladistic analysis. Since we were interested in the utility of male genitalia at higher-level relationships, we adopted a groundplan coding approach (Yeates 1995), and coded only those characters that were uniformly present in all members within each family (Table 3). The extent of intra-familiar variation of the included characters is briefly described below. All characters were coded as unordered and equally weighted. Characters 2–3, 4–5, 11–12, 13–15 and 18–21 were coded utilizing an additive binary coding technique. Inapplicable characters were coded as "–" and missing characters were coded as "?" Eumastacidae was used as a root. The matrix was created in WinClada (Nixon 2002).The description of characters is listed below. We generally followed the terminology of Dirsh (1956). Characters 0–6 were coded from epiphallus, characters 7–15 were coded from ectophallus and characters 16–25 were coded from endophallus.

0. Overall form of epiphallus: (0) shield-shaped; (1) disc-shaped; (2) bridge-shaped. The shield-shaped epiphallus is medium in size and partially covers ectophallus. This state is found in Eumastacidae (Fig. 2A), Pyrgomorphidae (Fig. 4C), Charilaidae (Fig. 4D) and Pamphagidae (Fig. 4E). The disc-shaped

Table 3. A character matrix based on male phallic complex.

	00000	00000	11111	11111	22222	2
	01234	56789	01234	56789	01234	5
Eumastacidae	000–0	–0000	0–00–	–100–	––001	0
Tanaoceridae	110–0	–0000	0–00–	–010–	––00?	0
Trigonopterygidae	110–0	–0100	0–010	01110	00010	0
Pneumoridae	110–0	–0000	0–00–	–010–	––022	0
Pyrgomorphidae	000–0	–0000	0–111	–110–	––001	0
Charilaidae	000–0	–1010	0–110	01110	10101	0
Pamphagidae	01100	–0010	10110	11110	11101	0
Pyrgacrididae	200–1	00010	1?110	??11?	?0101	0
Lentulidae	20101	01010	10110	0110–	––101	0
Lithidiidae	20111	10010	11110	01110	10101	0
Tristiridae	20111	01010	0–111	–1110	21101	0
Ommexechidae	200–1	01010	11110	1110–	––100	0
Romaleidae	20101	11010	10110	11110	11100	1
Acrididae	20111	11011	10110	*1111	11102	1

–, inapplicable data; ?, missing data; *, polymorphic data [0,1].

is comparatively larger than the shield-shaped epiphallus and completely covers ectophallus, and this state is found in basal families, Tanaoceridae (Fig. 2C), Trigonopterygidae (Fig. 4A) and Pneumoridae (Fig. 4B). Finally the bridge-shaped epiphallus is laterally elongated and does not cover ectophallus. This state is present in most families of Acridomorpha including Pyrgacrididae (not shown), Lentulidae (Fig. 4G), Lithidiidae (Fig. 4F), Tristiridae (Fig. 4H), Ommexechidae (Fig. 4I), Romaleidae (Fig. 4J) and Acrididae (Fig. 4K).

1. Median projection of epiphallus: (0) absent; (1) present.These are small projections that are in the middle of epiphallus, as seen in Tanaoceridae (Fig. 2C), Trigonopterygidae (Fig. 4A), Pneumoridae (Fig. 4B) and Pamphagidae (Fig. 4E).
2. Ancorae of epiphallus: (0) absent; (1) present. These are a pair of projections at each side of the anterior part of epiphallus.
3. Of 2(1), the shape of ancorae: (0) lobiform; (1) angular. When ancorae are present, they apex could be blunt and lobiform as in Pamphagidae (Fig. 4E), Lentulidae (Fig. 4G) and Romaleidae (Fig. 4J) or acute and angular as in Lithidiidae (Fig. 4F), Tristiridae (Fig. 4H) and Acrididae (Fig. 4K).
4. Lophi of epiphallus: (0) absent; (1) present. These are a pair of projections at each side of the posterior part of the structure.
5. Of 4(1), the shape of lophi: (0) hook-shaped; (1) lobiform. The lophi can be hook-shaped and acute as in Lentulidae (Fig. 4G), Tristiridae (Fig. 4H) and Ommexechidae (Fig. 4I) or lobiform and blunt as in Lithidiidae (Fig. 4F), Romaleidae (Fig. 4J) and Acrididae (Fig. 4K).
6. Oval sclerites of epiphallus: (0) absent; (1) present. These are a pair of extra sclerites located at the sides in the anterior portion of epiphallus.

7. Position of the entire phallic complex: (0) normal; (1) reverse. In the phallic complex in Trigonopterygidae (Fig. 2D), the dorsal side is turned ventrally, with the aedeagus directed towards the anterior end of the body and epiphallus in a ventral position.
8. Level of sclerotization in ectophallus: (0) partly sclerotized (Fig. 2A–D); (1) fully sclerotized (Fig. 3B–I).
9. Valves of cingulum: (0) absent; (1) present. In Acrididae, there is one pair of valves of cingulum that matches the pair of apical valves of aedeagus (Fig. 3I).
10. Rami of cingulum: (0) absent; (1) present. These are lateral projections at the anterior part of the cingulum that envelop the aedeagus.
11. Of 10(1), the shape of rami: (0) wide; (1) narrow. When present, the shape of rami could be wide (more or less as long as the width) or narrow (longer than its width).
12. Zygoma of cingulum: (0) absent; (1) present. It is located in the middle of the cingulum when viewed from dorsally. It is absent in some basal families (Fig. 2A–D), but present in Acridoidea (Fig. 3A–I).
13. Apodemes of cingulum: (0) absent; (1) present. These are dorsally located above the zygoma. These apodemes can be either absent as in the families Eumastacidae (Fig. 2A), Tanaoceridae (Fig. 2C) and Pneumoridae (Fig. 2B) or present as in the rest of Acridomorpha.
14. Of 13(1), the level of differentiation in the apodemes of cingulum: (0) differentiated; (1) not differentiated.
15. Of 14(1), the shape of the apodemes of cingulum: (0) long; (1) short. When the apodemes are well differentiated, their shape can be elongated as in Trigonopterygidae (Fig. 2D), Charilaidae (Fig. 3D), Lentulidae (Fig. 3E), Lithidiidae (Fig. 3D) and Acrididae (Fig. 3I) or short and compact as in Pamphagidae (Fig. 3C), Ommexechidae (Fig. 3G) and Romaleidae (Fig. 3H).

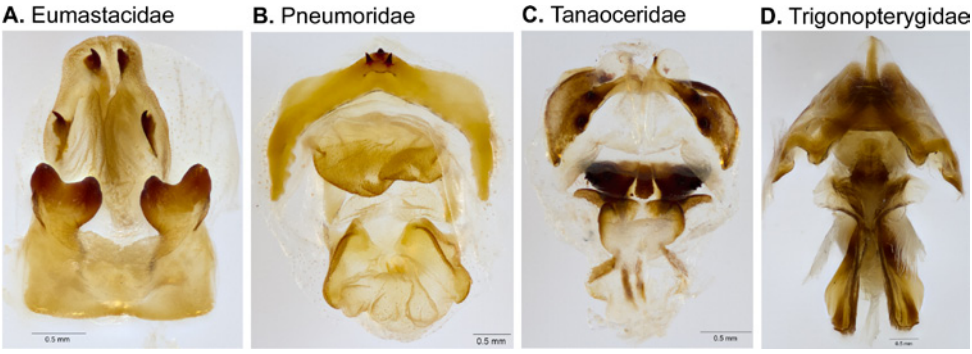

Fig. 2. The male phallic complex of basal acridomorph families. Epiphallus of Triginoterygidae (D) is not shown. This figure is published in colour in the online version of this journal, which can be accessed via http://booksandjournals.brillonline.com/content/1876312x.

16. Level of sclerotization in endophallus: (0) membranous; (1) highly sclerotized.
17. Number of sclerites in penis: (0) one; (1) two. In the case of the family Eumastacidae, the penis is composed of only one sclerite, whereas in the remaining families, the penis is divided in two sclerites.
18. Sclerite division in endophallus: (0) absent; (1) present. Endophallus is either undivided or divided in two (basal valves and apical valves).
19. Of 18(1), the shape of basal valves of penis: (0) robust; (1) narrow. Among the families with the divided endophallus, the shape of the basal valves could be robust as in Trigonopterygidae (Fig. 2D), Charilaidae (Fig. 3B), Pamphagidae (Fig. 3C), Lithidiidae (Fig. 3D), Tristiridae (Fig. 3F) and Romaleidae (Fig. 3H) or narrow in the case of Acrididae (Fig. 3I).
20. Of 18(1), the shape of apical valves of penis: (0) bilobate (Fig. 2D); (1) narrow (Fig. 3B–D); (2) wide (Fig. 3F).
21. Of 18(1), connection between basal valves and apical valves: (0) disconnected; (1) articulated. In Pamphagidae, Tristiridae, Romaleidae and Acrididae, the connection between the two valves is articulated like a hinge.
22. Gonopore developed as a constriction separating the ejaculatory sac from the spermatophore sac: (0) absent; (1) present. The ejaculatory sac and the spermatophore sac can bea continuous tube without division as in Eumastacidae, Tanaoceridae, Trigonopterygidae, Pneumoridae and Pyrgomorphidae or there can be a constriction, which divides the two sacs as in the rest of Acridoidea.
23. Position of ejaculatory sac in endophallus: (0) ventral; (1) dorsal; (2) transverse.
24. Position of spermatophore sac in endophallus: (0) ventral; (1) dorsal (as in Trigonopterygidae); (2) transverse (as in Pneumoridae).
25. Gonopore processes in endophallus: (0) absent; (1) present. Usually gonopore is a simple structure but in the case of Romaleidae and Acrididae there are processes.

We analyzed the character matrix in two ways. First, in order to assess the level of phylogenetic signal in the male phallic structures as interpreted by the previous taxonomists, we reconstructed a "male genitalia tree" based solely on the phallic structures in a parsimony framework in NONA (Goloboff 1995) using the following commands: rs 0; hold 10000; mult* 1000; best. Due to the relatively small size of the data set, TBR and SBR search methods were sufficient to find the most parsimonious tree. To assess nodal support, we calculated Bremer support (Bremer 1994) in NONA. Based on the most parsimonious tree, we optimized each male genital character on to the topology using ACCTRAN, DELTRAN and unambiguous and recorded consistency index (CI) and retention index (RI). Second, in order to independently test the evolution of male phallic structures, we also optimized the same characters on to the topology based on mitochondrial genome data and calculated CI and RI of each genital character. We compared and contrasted the results of character optimization between two topologies to identify taxonomic utility of each character in the higher-level classification of Acridomorpha.

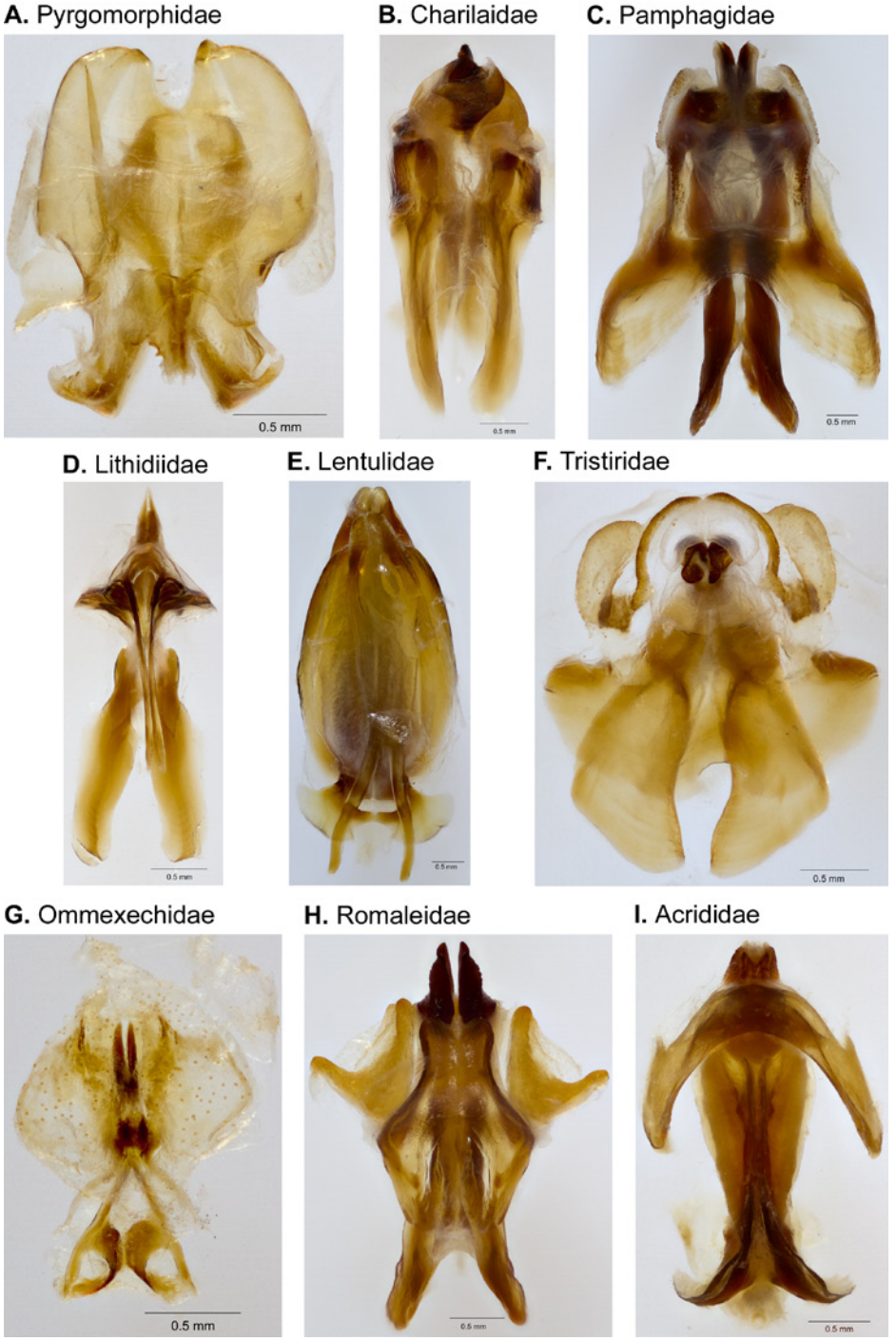

Fig. 3. The male phallic complex (ectophallus and endophallus) of representative families of Acridomorpha. This figure is published in colour in the online version of this journal, which can be accessed via http://booksandjournals.brillonline.com/content/1876312x.

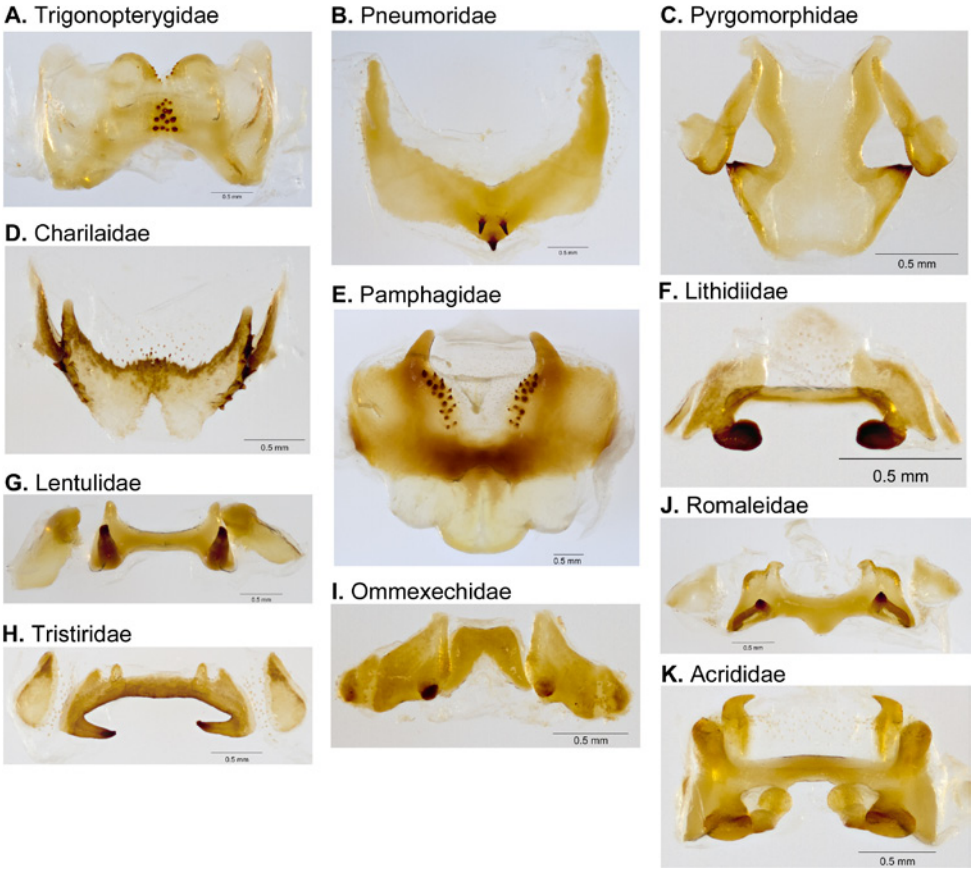

Fig. 4. Epiphalli of representative families of Acridomorpha. This figure is published in colour in the online version of this journal, which can be accessed via http://booksandjournals.brillonline.com/content/1876312x.

Results

Male genitalia tree

The parsimony analysis based on the male phallic structures found a single most parsimonious tree (Fig. 5A, L = 47, CI = 0.63, RI = 0.73). At the level of superfamily, we recovered the following relationships based on male genitalia: (Eumastacoidea ((Trigonopterygoidea (Tanaoceroidea, Pneumoroidea)) (Pyrgomorphoidea, Acridoidea))). The largest superfamily Acridoidea was recovered as a monophyletic group. Within Acridoidea, the following relationships were recovered: (Charilaidae (Pyrgacrididae (Lithidiidae (Tristiridae (Lentulidae (Pamphagidae (Ommexechidae (Romaleidae, Acrididae))))))))). When compared with the topology based on mitochondrial genome data (Fig. 5B), the male genitalia tree was congruent in findings that Acridoidea was monophyletic and that it was sister to Pyrgomorphoidea. However, both disagreed on the remaining higher-level relationships as well as internal relationships within Acridoidea.

Character optimization on the male genitalia tree

Of the 26 characters, six characters were phylogenetically uninformative for grouping because they were either autapomorphic or plesiomorphic and there were: characters 7, 9, 17, 19, 20 and 23. Seven characters were shown to be uncontroverted synapomorphies (Fig. 5A) and were highly informative in grouping different clades and they were: character 8(1), fully sclerotized ectophallus which grouped Acridoidea; character 12(1), the presence of zygoma of cingulum which grouped Pyrgomorphoidea and Acridoidea; character 15(1), short apodemes of cingulum which grouped Pamphagidae, Ommexechidae, Romaleidae and Acrididae; character 16(0), membranous endophallus, which grouped Tanaoceridae and Pneumoridae; character 21(1), articulated connection between basal valves and apical valves of penis, which grouped Tristiridae, Lentulidae, Pamphagidae, Ommexechidae, Romaleidae and Acrididae; character 22(1), gonopore developed as a constriction separating the ejaculatory sac from the spermatophore sac, which grouped Acridoidea; and character 25(1), the presence of gonopore processes in endophallus, which grouped Romaleidae and Acrididae. Other characters varied in their level of homoplasy (CI) and synapomorphy (RI).

Character optimization on the molecular tree

When the male phallic characters were optimized on to the molecular tree, we found that four characters were uncontroverted synapomorphies (Fig. 5B), three of which were also found in the character optimization on the male genitalia tree and they were: characters 8(1), 12(1) and 22(1). The fourth uncontroverted synapomorphy was character 4(1), the presence of lophi in epiphallus, which grouped Pyrgacrididae, Lentulidae, Lithidiidae, Tristiridae, Acrididae, Ommexechidae and Romaleidae. When CI and RI of each character optimized on to the male genitalia tree and the molecular tree were compared with each other, we found that of the twenty phylogenetically informative characters, ten characters had the same values between the two, nine characters had lower values and one character had higher values when optimized onto the molecular tree (Table 4).

Discussion

How much phylogenetic signal do genital structures possess and are they free of homoplasy?

A cladistic analysis based on morphological characters often relies on a researcher's own interpretation of homology across taxa, but the interpretation is subject to change in light of new data. However, if enough weight is given to a certain type of data, the analysis will likely result in groupings biased by these data, regardless of an otherwise good phylogenetic signal from other characters. Although none of the earlier taxonomists of Acridomorpha conducted a proper cladistic analysis, their phylogenetic hypotheses and the resulting higher-level classification schemes were influenced by a type of character weighting because of the preconceived notion that male genitalia were the most informative character system (Roberts 1941; Dirsh 1956).

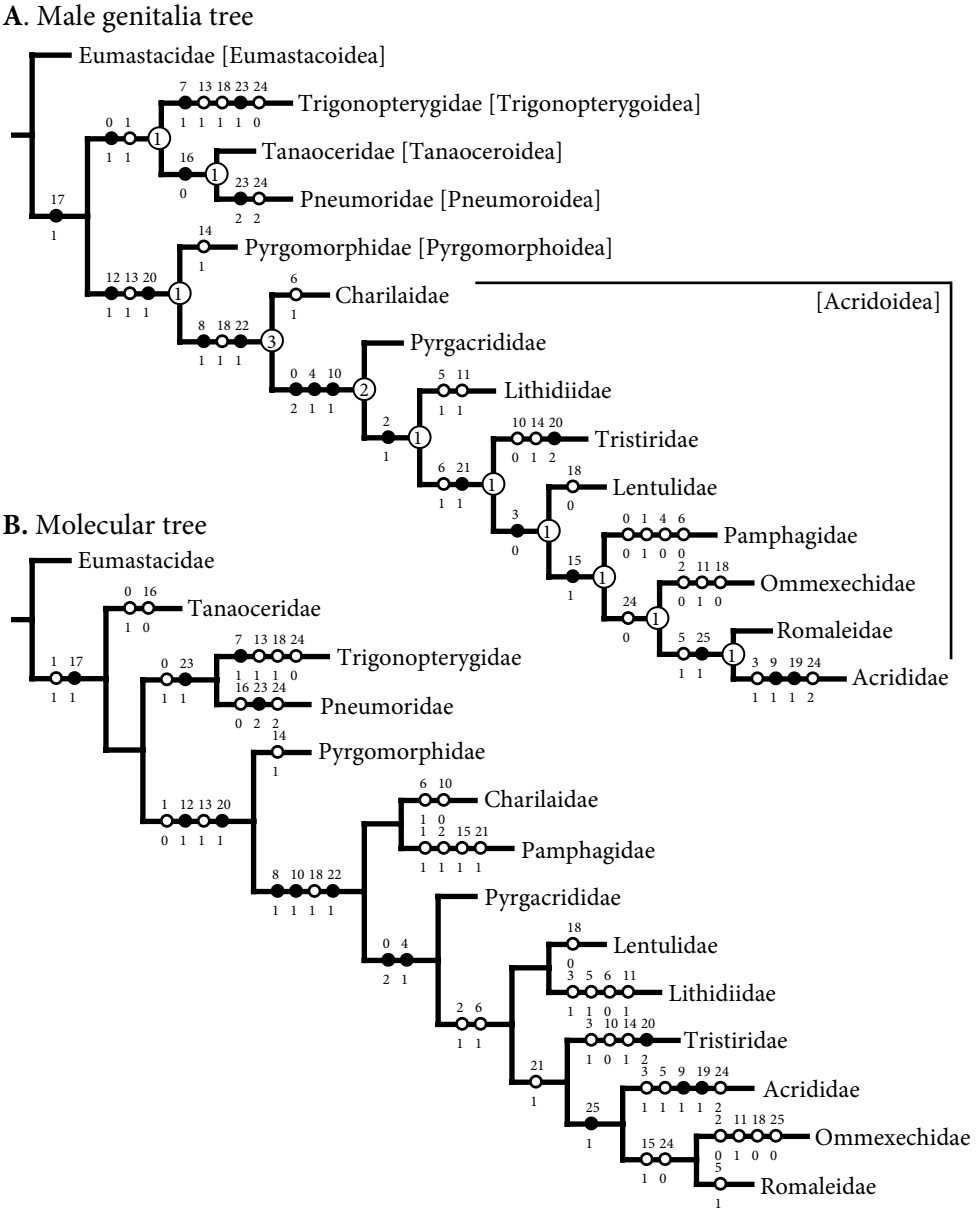

Fig. 5. (A) The single most parsimonious tree recovered from male phallic structures (L = 47, CI = 0.63, RI = 0.73); (B) A family-level phylogeny deduced from mitochondrial genome data (Leavitt et al. 2013). On both trees, 26 characters and their states are optimized. Black circles represent uncontroverted apomorphies and white circles represent homoplasies. The numbers above and below each circle represent the character number and its state, respectively. The numbers on the nodes in the male genitalia tree are Bremer support values.

Table 4. Results of character optimization of male phallic characters on to the male genitalia tree and the molecular tree.

Character	Male genitalia tree		Molecular tree	
	CI	RI	CI	RI
0	0.66	0.80	0.66	0.80
1	0.50	0.66	0.33	0.33
2	0.50	0.80	0.33	0.60
3	0.50	0.50	0.33	0.00
4	0.50	0.83	1.00	1.00
5	0.50	0.50	0.33	0.00
6	0.33	0.60	0.33	0.60
7	uninf.	uninf.	uninf.	uninf.
8	1.00	1.00	1.00	1.00
9	uninf.	uninf.	uninf.	uninf.
10	0.50	0.83	0.33	0.66
11	0.50	0.00	0.50	0.00
12	1.00	1.00	1.00	1.00
13	0.50	0.50	0.50	0.50
14	0.50	0.00	0.50	0.00
15	1.00	1.00	0.50	0.50
16	1.00	1.00	0.50	0.00
17	uninf.	uninf.	uninf.	uninf.
18	0.25	0.40	0.25	0.40
19	uninf.	uninf.	uninf.	uninf.
20	uninf.	uninf.	uninf.	uninf.
21	1.00	1.00	0.50	0.66
22	1.00	1.00	1.00	1.00
23	uninf.	uninf.	uninf.	uninf.
24	0.50	0.33	0.50	0.33
25	1.00	1.00	0.50	0.00

CI, consistency index; RI, retention index; uninf., uninformative character.

In this study, we examined the amount of phylogenetic signal present in male genitalia by reconstructing a phylogeny based on male genitalia and compared it to an independent and robust phylogeny based on molecular data. The level of overall phylogenetic signal can be indirectly inferred from examining measures of fit (CI and RI) (Sanderson & Donoghue 1989, 1996; Song & Bucheli 2010) and we found that the phallic characters had relatively high measures of fit (CI = 0.63, RI = 0.73), suggesting that they had inherently strong phylogenetic signal. However, the measures of fit are analysis-dependent and there needs to be an independent assessment of the characters. If male genitalia indeed had strong and accurate phylogenetic signal, then we would expect to find some level of congruence between the male genitalia tree and the molecular tree. Any incongruence between the two trees could simply indicate differences in the level of phylogenetic signal in male genitalia and mitochondrial genomes, but it could also indicate possible biases in either character systems. Both the male genitalia tree and the molecular tree recovered a sister relationship between Pyrgomorphoidea

and Acridoidea as well as monophyletic Acridoidea. Supporting these relationships are three uncontroverted synapomorphies that the character optimization analyses of phallic structures on both trees share: the presence of zygoma of cingulum, fully sclerotized ectophallus, and gonopore developed as a constriction separating the ejaculatory sac from the spermatophore sac. The remaining 23 characters, however, supported groupings that were not supported by the molecular tree.

The male genitalia tree, which was largely based on literature data, resulted in a topology that is also highly incongruent with the previous phylogenetic hypotheses by Amédégnato (1976), Dirsh (1956), Eades (2000) and Roberts (1941). For example, while a close relationship between Pamphagidae and Charilaidae was consistently found across different classification schemes (Song 2010), the male genitalia tree did not group the two families together. Similarly, both Dirsh (1956) and Amédégnato (1976) considered Pyrgomorphidae and Lentulidae to the most closely related, but the present analysis did not find this relationship. One possible explanation for these discrepancies would result from the differences in the way the previous taxonomists deduced their phylogenetic hypotheses and how we reconstructed the male genitalia tree. Dirsh (1956) worked in the framework of evolutionary taxonomy, Amédégnato (1976) used phenetics, and Eades (2000) presented a synthesis of previous studies rather than a formal analysis. In a cladistic analysis, especially when characters are coded without weighting, each homology statement is allowed to 'compete' with each other during phylogenetic reconstruction, and some characters are shown to be homoplasious as a result. Of the 26 characters, we found that 13 characters had CI ranging between 0.33 and 0.66 (Table 4), which would suggest that some of the characters of male genitalia by the previous taxonomists used must have evolved multiple times.

Our finding strongly suggests that the male phallic structures of Acridomorpha are not free of homoplasy when used for phylogenetic analyses. The initial notion that internal male genitalia would be less influenced by external environment may still hold true (Roberts 1941), but recent developments in the study of evolution of male genitalia strongly suggest that male genitalia indeed evolve rapidly because they are under strong sexual selection (Eberhard 1985, 2004; Arnqvist & Rowe 2002; Hosken & Stockley 2004). Then, are male genitalia not useful for understanding the higher-level relationships within Acridomorpha? The answer is resounding 'no' because we found that globally homoplasious characters could be locally synapomorphic and this pattern has been found in many insect groups (Song & Bucheli 2010). For example, when the male phallic structures were optimized on to the molecular tree, we found that 10 controverted synapomorphies were supporting various clades across Acridomorpha. Some of the notable ones included the apodemes of cingulum, which grouped Pyrgomorphoidea and Acridoidea, but also independently evolved in Trigonopterygidae, and articulated connection between basal valves and apical valves of penis, which grouped Tristiridae, Acrididae, Ommexechidae and Romaleidae, but also evolved in Pamphagidae. This pattern suggests that despite the frequent homoplasies, the male phallic structures of Acridomorpha in fact contain useful information for the higher-level classification.

Do male genitalia evolve from simple to complex in Acridomorpha?

The idea that complexity increases in evolution is pervasive, but there is surprisingly little evidence supporting this idea (McShea 1991). When applied to the male phallic structures of Acridomorpha, undifferentiated and membranous genitalia have been considered "simple" while differentiated and sclerotized genitalia have been considered "complex." Likewise, families that have the simple genitalia have been considered "primitive" and those that have the complex genitalia have been considered "advanced" (Dirsh 1956). Among the characters included in the analysis, two characters were related to the level of sclerotization in ectophallus (character 8) and endophallus (character 16). In both the male genitalia tree and the molecular tree, fully sclerotized ectophallus was an uncontroverted synapomorphy that grouped Acridoidea and other basal superfamilies had only partly sclerotized structures, suggesting that the polarity of the character evolution for ectophallus would point to the increasing level of sclerotization. However, in endophallus, Tanaoceridae and Pneumoridae were the only families that had the membranous structure, but these two families were not at the very base of Acridomorpha phylogeny, suggesting that sclerotization might have been lost in them, possibly independently. In this case, the idea of increasing complexity is not supported.

Because the concepts of simplicity and complexity are abstract, relative and difficult to quantify, the notion that something is simple or complex is often based on gestalt, which can be highly variable and context-dependent (McShea 1991). Based on the examination of the male phallic complex of representative families of Acridomorpha using high-resolution macrophotography (Figs 2–4), we tried to determine which family had the most complex or the simplest male genitalia, but we were unable to reach a conclusion because each family had the "complex" phallic structures in its own right. Similarly, it is difficult to consider the acridomorph groups that have relatively undifferentiated and membranous genitalia as simple or less evolved because these concepts are abstract and relative. For example, Pneumoridae is characterized by undifferentiated phallic structures and thus has been considered primitive by many authors (Dirsh 1956; Amédégnato 1976). However, the family is well known for its unique femoroabdominal stridulatory mechanism (Dirsh 1965) and males have a swollen abdomen that can amplify their calls for a long distance signal (van Staaden & Römer 1997). Perhaps the male phallic structures of Pneumoridae lost the level of sclerotization because sexual selection has worked strongly on the pre-copulatory acoustic courtship mechanism, thereby lifting selective pressure off male genitalia. Therefore, we challenge the idea that male genitalia of Acridomorpha evolve from simple to complex and argue that the polarity of character transformation has be to studied in the context of phylogenetic analyses.

Concluding remarks

When earlier taxonomists discovered the taxonomic value of male phallic structures for the first time (Roberts 1941), they must have felt that they found a silver bullet that could confidently solve difficult phylogenetic problems. In many aspects, the study of

male genitalia revolutionized the higher-level classification of Acridomorpha (Song 2010), but we show that even these characters are often homoplasious and might have misled the taxonomists into making incorrect phylogenetic conclusions. With new molecular data, we are now capable of independently testing the taxonomic and phylogenetic utility of these structures, and we show that the male genitalia of Acridomorpha can be useful for higher-level classification, but need to be used with caution.

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