

Phylogenetic patterns of leg morphometric traits in vespid wasps (Hymenoptera: Vespidae)

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Abstract

Vespid wasps use legs primarily for locomotion and in contexts such as grooming, handling of food and nest materials. Studies on leg form and function are relatively scarce. We analysed measurements of legs of 127 species of all genera of Polistinae, and of 18 species of other subfamilies, with the intent of investigating the occurrence and phylogenetic locations of morphometric patterns. Body-size variation across Polistinae was determinant as to the absolute dimensions of legs, but allometric effects are generally small when comparing proportional length differences between differently sized species. Quite differently, size-independent shape aspects are notably distinct as expressed by regression residuals and are clearly phylogenetically structured. Most distinctive are the very long mid and hind legs (and simultaneously exceedingly short forelegs) of *Mischocyttarus* species, and the comparatively shorter legs of some Epiponini. We found that forelegs have evolved with relative independence of the mid and hind legs, which is probably related to functions associated with the transport and manipulation of food and materials. Most examined solitary species showed mid and hindlegs shorter than most social polistines and vespines. Forefemur shape in some solitary species (euparagiines, masarines and some eumenines) may be functionally related to fossorial habits.

KEYWORDS

evolutionary allometry, morphological integration, phylogenetic regression, size-independent shape, wasp legs

1 | INTRODUCTION

Insect legs are articulated appendages that perform a range of functions primarily related to the organism's movements through the environment and are often involved in the collection and manipulation of food (including prey capture) and other habitat resources (Chapman, 1998; Cruse et al., 2009; Grimaldi & Engel, 2005). In Hymenoptera, legs play various

additional roles such as self-cleaning (grooming), preparation of nests and temporary storage of food (pollen) in bees (Basibuyuk & Quicke, 1999; Evans & Eberhard, 1970; Michener, 2007). In many groups, legs show considerable shape modifications, often in the form of enlargement of some parts (e.g., the forelegs of female Sclerogibbidae and some Rhopalosomatidae, and the hindlegs of Chalcididae and some Stephanidae, the pollen-storing modifications in some bees, and the

enlarged posterior tibiae of male Euglossini; Lensky et al., 1985; Rafael et al., 2012). Very common are the rake-like structures formed by spine-shaped setae of legs, which are used in several groups of aculeates for substrate excavation (Bohart & Menke, 1976; Evans & Eberhard, 1970; Fernández & Sharkey, 2006).

The literature on the general biology of the vespid wasps generally considers the legs to be unspecialized structures (Duncan, 1939; Edwards, 1980; Spradbery, 1973). Functions are primarily related to short-range locomotion by walking on nests, vegetation, spider webs and diverse types of substrates. Legs also properly position the body in contexts such as fighting, feeding larvae, exchanging materials with nest mates, mating, ovipositing and stinging. Equally important, legs play specific roles in individual grooming and help the mouthparts in the gathering, transportation, and manipulation of food and materials (Evans & Eberhard, 1970).

Morphological and sociobiological studies have occasionally treated in more detail the roles of legs in the behavioural actions of social vespids, as in Duncan (1939), West-Eberhard (1969) and Jeanne (1972). The latter paper on *Mischocyttarus drewseni* is rich with precise descriptions of the roles of wasps' legs (and some leg parts). Jeanne's description of wasps robbing dead insects from spider webs is very detailed, and author's hypothesis relating tarsal lobe asymmetry of *Mischocyttarus* species to their capacity to climb spider webs to steal prey is notable in the context of the study of correlations between form and function in wasps.

A few studies (Frantsevich & Gorb, 2002, 2004) have investigated the structure and functioning of the distal tarsal elements of the legs of some vespine and polistine species (*Vespa crabro* L.; *Vespula germanica* (F.), *V. vulgaris* (L.), *Polistes gallicus* (L.)). Gladun and Gorb (2007) studied the general morphology of the distal tarsal and pretarsal leg elements in various insects, including several Hymenoptera and the vespid *Eumenes coarctatus* (L.), and performed experiments on the locomotory ability of these insects on some types of surfaces.

Despite the absence of extreme modifications in the shape of the legs of vespids (Figure 1), preliminary experience with the morphology of these wasps has shown that some patterns of leg length variation appear sufficiently distinct to warrant further investigation of their possible functional or behavioural consequences. In this paper, we take a first step in that direction, presenting and analysing length measurements of four segmental parts of the legs (coxa, femur, tibia and tarsus). The trochanter (small intercalary segment between coxa and femur) was hardly variable in proportions in preliminary examinations and was not included among the measured leg parts (Duncan, 1939, on trochanters of *V. pensylvanica* noted ... "are small and

closely articulated with the femora but exhibit no noteworthy modifications"). Our sample comprised 127 species of all genera of Polistinae (Figure 1); 3 species of Vespinae; 2 species of Stenogastrinae; and 13 species of solitary Euparagiinae, Masarinae and Eumeninae. The main aim of the study was to determine the strength (or distinctiveness) and phylogenetic locations of purported shape patterns.

2 | MATERIALS AND METHODS

2.1 | Data summary

Names of taxa and indications of the datasets (D1, D2) in which a species is a part are presented in Table 1 (see also



FIGURE 1 Eusocial Polistinae on nests in the field (not to the same scale): (a) *Mischocyttarus annulatus*; (b) *Polistes goeldii*; (c) *M. suzannae*; (d) *Polybia affinis*; (e) *Synoeca virginea*; (f) *Brachygastra bilineolata*; (g) *Protopolybia diligens*; (h) *Pseudopolybia difficilis*; (i) *Polybia rejecta*. Scale bars ca. 10 mm.

TABLE 1 List of names of studied vespids with respective abbreviations, and the indication of the data sets (D1, D2) in which a species is a part; species in data set D1 marked with a “C” also had the coxae measured.

Abbr.	Taxon name	D1	D2	Acron.	Taxon name	D1	D2
agan	<i>Agelaia angulata</i> (Fabricius 1804)	XC	X	mikall	<i>M. kallindusflore</i> Borges & Silveira 2019	X	
agcj	<i>A. cajennensis</i> (Fabricius 1798)	X		milat	<i>M. latior</i> (Fox 1898)	XC	
agct	<i>A. constructor</i> (de Saussure 1854)	X		mimt	<i>M. metathoracicus</i> (de Saussure 1854)	X	
agflpn	<i>A. flavipennis</i> (Ducke 1905)	XC		miprut	<i>M. nr. prominulus</i> 1	X	
agfv	<i>A. fulvofasciata</i> (Degeer 1773)	XC	X	mipran	<i>M. annulatus</i> Richards, 1978	XC	
aglb	<i>A. lobipleura</i> (Richards, 1978)	XC		mipun	<i>M. punctatus</i> (Ducke 1904)	XC	
agmy	<i>A. myrmecophila</i> (Ducke 1905)	XC		mirfd	<i>M. rufidens</i> (de Saussure 1854)	XC	X
agpl	<i>A. pallipes</i> (Olivier 1792)	XC	X	mirot	<i>M. rotundicollis</i> (Cameron 1912)	XC	
agtes	<i>A. testacea</i> (Fabricius 1804)	XC		mishnk	<i>M. schunkei</i> Zikán 1949	XC	
anpl	<i>Angiopolybia pallens</i> (Lepeletier 1836)	XC	X	micss	<i>M. cassununga</i> (von Ihering 1903)	XC	
anpr	<i>An. paraensis</i> (Spinola 1851)	XC	X	misur	<i>M. surinamensis</i> (de Saussure 1854)	XC	
apar	<i>Apoica arborea</i> de Saussure 1854	X		mtci	<i>Metapolybia cingulata</i> (Fabricius 1804)	XC	
appd	<i>Ap. pallida</i> (Olivier 1792)	XC		mtdc	<i>Met. docilis</i> Richards, 1978	XC	
appl	<i>Ap. pallens</i> (Fabricius 1804)	X		mtng	<i>Met. nigra</i> Richards, 1978	XC	
aphor	<i>Ap. thoracica</i> du Buysson 1906	X		mtrf	<i>Met. rufata</i> Richards, 1978	XC	
astr	<i>Asteloeca traili</i> (Cameron 1906)	XC	X	mtsf	<i>Met. sufusa</i> (Fox 1898)	XC	
belj	<i>Belonogaster juncea</i> (Fabricius 1781)	XC		ncch	<i>Nectarinella championi</i> (Dover 1925)	X	
besp	<i>Belonogaster</i> sp.	X		pain	<i>Parapolybia indica</i> (de Saussure 1854)	XC	
brfist	<i>Brachygastra fistulosa</i> Naumann 1968	X		pbml	<i>Polybioides melainus</i> (Meade-Waldo 1911)	X	
brbi	<i>B. bilineolata</i> Spinola 1841	XC		pbsp	<i>Polybioides</i> sp.	X	
brlc	<i>B. lecheguana</i> (Latreille 1824)	XC	X	plbic	<i>Polistes bicolor</i> Lepeletier 1836	XC	
brsc	<i>B. scutellaris</i> (Fabricius 1804)	XC	X	plcd	<i>P. canadensis</i> (Linnaeus 1758)	XC	X
cgen	<i>Chartergellus nigerrimus</i> Richards, 1978	X	X	plcx	<i>P. carnifex</i> (Fabricius 1775)	XC	
cgep	<i>C. punctatior</i> Richards, 1978	XC		plhbre	<i>P. “hebraeus”</i> [= <i>olivaceus</i> (Degeer 1773)]	XC	
cgif	<i>Charterginus fulvus</i> Fox 1898	XC		plhum	<i>P. humilis</i> (Fabricius 1781)	XC	
chgl	<i>Chartergus globiventris</i> de Saussure 1854	X		plsat	<i>P. satan</i> Bequaert 1940	X	
chmt	<i>Ch. metanotalis</i> Richards, 1978	XC	X	ploc	<i>P. occipitalis</i> Ducke 1904	X	
cyap	<i>Clypearia apicipennis</i> (Spinola 1851)	XC		plpf	<i>P. pacificus</i> Fabricius 1804	XC	
cydk	<i>Cl. duckei</i> Richards, 1978	X		plplt	<i>P. dominula</i> (Christ 1791)	XC	
cysu	<i>Cl. sulcata</i> (de Saussure 1854)	XC	X	plsu	<i>P. subsericeus</i> de Saussure 1854	X	X
cywy	<i>Cl. weyrauchi</i> Richrads 1978	XC		plvr	<i>P. versicolor</i> (Olivier 1792)	XC	X
epta	<i>Epipona tatua</i> (Cuvier 1797)	XC		pobru	<i>Polybia brunnea</i> (Curtis 1844)	X	
ledo	<i>Leipomeles dorsata</i> (Fabricius 1804)	XC	X	pobt	<i>Po. bistrata</i> (Fabricius 1804)	XC	X
leps	<i>L. spilogastra</i> (Cameron 1912)	XC		pocc	<i>Po. occidentalis</i> (Olivier 1792)	XC	X
miadlp	<i>Mischocyttarus adolphi</i> Zikán 1935	XC		poch	<i>Po. chrysothorax</i> (Lichtenstein 1796)	XC	
miang	<i>M. angulatus</i> Richards 1945	XC		pocctx	<i>Po. catillifex</i> Mobius 1856	XC	
miash	<i>M. asahi</i> Borges & Silveira 2019	X		podl	<i>Po. dimidiata</i> (Olivier 1792)	XC	X
miart	<i>M. artifex</i> Ducke 1914	X		podm	<i>Po. dimorpha</i> Richards, 1978	XC	
mibuy	<i>M. buysoni</i> (Ducke 1906)	XC		podp	<i>Po. depressa</i> (Ducke 1905)	XC	
micb	<i>M. cerberus</i> Ducke 1918	XC		poig	<i>Po. ignobilis</i> (Haliday 1836)	XC	

(Continues)

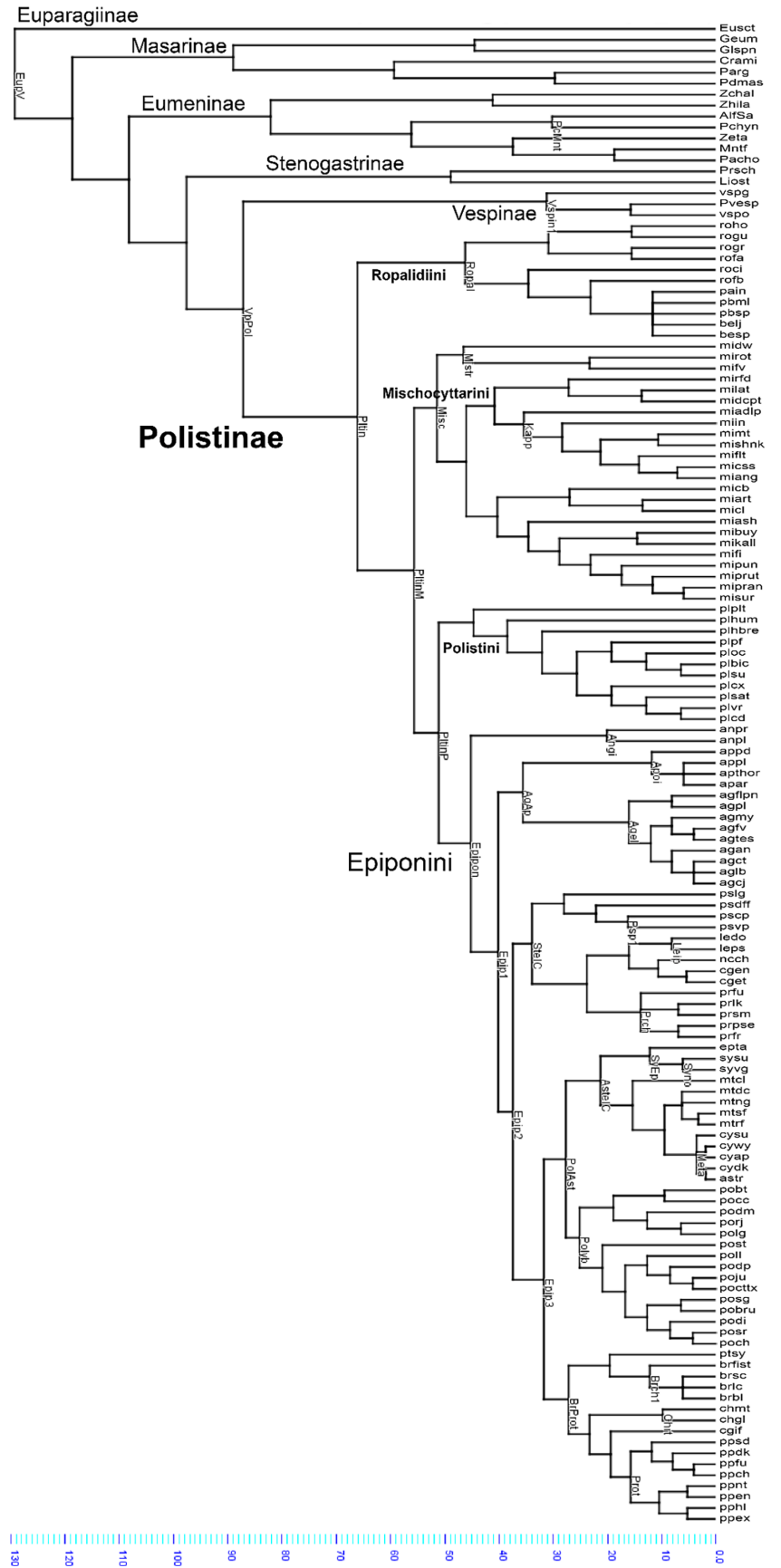
TABLE 1 (Continued)

Abbr.	Taxon name	D1	D2	Acron.	Taxon name	D1	D2
micl	<i>M. collaris</i> (Ducke 1904)	XC		poju	<i>Po. jurinei</i> de Saussure 1854	XC	
midcpt	<i>M. deceptus</i> (Fox 1897)	XC	X	poll	<i>Po. liliacea</i> (Fabricius 1804)	XC	
midw	<i>M. drewseni</i> de Saussure 1857	XC	X	porj	<i>Po. rejecta</i> (Fabricius 1798)	XC	X
mifi	<i>M. filiformis</i> (de Saussure 1854)	XC		posg	<i>Po. singularis</i> Ducke 1909	XC	
miflt	<i>M. flavitarsis</i> (de Saussure 1854)	XC		posr	<i>Po. sericea</i> (Olivier 1792)	XC	X
mifv	<i>M. flavicans</i> (Fabricius 1804)	XC		post	<i>Po. striata</i> (Fabricius 1787)	X	
miin	<i>M. injucundus</i> (de Saussure 1854)	XC		ppch	<i>Protopolybia chartergoides</i> (Gribodo 1892)	XC	X
ppdk	<i>Pr. duckeianus</i> Richards, 1978	X		vspg	<i>Vespula germanica</i> (Fabricius 1793)	XC	
ppem	<i>Pr. emortualis</i> (de Saussure 1855)	XC		vspo	<i>Vespa orientalis</i> Linnaeus 1771	XC	
ppex	<i>Pr. exigua</i> (de Saussure 1854)	XC		Pvesp	<i>Provespa anomala</i> (de Saussure 1854)	XC	
ppfu	<i>Pr. fuscatus</i> (Fox 1898)	XC		Liost	<i>Liostenogaster flavolineata</i> (Cameron 1902)	XC	
pphl	<i>Pr. holoxantha</i> (Ducke 1904)	XC		Prsch	<i>Parischnogaster striatula</i> (du Buysson 1905)	XC	
ppnt	<i>Pr. nitida</i> (Ducke 1904)	X		Eusct	<i>Euparagia scutellaris</i> Cresson 1879	X	
ppsd	<i>Pr. sedula</i> (de Saussure 1854)	XC		Crami	<i>Ceramius bicolor</i>	XC	
prfr	<i>Parachartergus fraternus</i> (Gribodo 1892)	XC		Geum	<i>Gayella eumenoides</i> Spinola 1851	XC	
prfu	<i>Pa. fulgidipennis</i> (de Saussure 1854)	XC		Glspn	<i>Gayella luispenai</i> Willink & A. de Toledo 1979	XC	
prlk	<i>Pa. lenkoi</i> Richards, 1978	XC		Parg	<i>Paragia decipiens</i>	XC	
prpse	<i>Pa. pseudapicalis</i> Willink 1959	XC		Pdmas	<i>Pseudomasaris vespoides</i> (Cresson 1863)	XC	
prsm	<i>Pa. smithii</i> (de Saussure 1854)	X		AlfSa	<i>Alphamenes</i> sp.	XC	
pscp	<i>Pseudopolybia compressa</i> (de Saussure 1854)	XC		Mntf	<i>Montezumia ferruginea</i> de Saussure 1852	XC	
psdff	<i>Ps. difficilis</i> (Ducke 1905)	X		Pacho	<i>Pachodynerus</i> sp.	XC	
pslg	<i>Ps. langi</i> Bequaert 1944	X		Pchyn	<i>Pachymenes novarae</i> de Saussure 1867	XC	
psvp	<i>Ps. vespiceps</i> (de Saussure 1863)	XC		Zeta	<i>Zeta argillaceum</i> (Linnaeus 1758)	XC	
ptsy	<i>Protonectarina sylveirae</i> (de Saussure 1854)	X		Zchal	<i>Zethus chalybeus</i> de Saussure 1852	XC	
roci	<i>Ropalidia cincta</i> (Lepeletier 1836)	X		Zhila	<i>Zethus hilarianus</i> de Saussure 1855	XC	
rofa	<i>Ro. fasciata</i> (Fabricius 1804)	XC		apflvs	<i>Apoica flavissima</i> van der Vecht 1972		X
rofb	<i>Ro. flavobrunnea</i> van der Vecht 1962	X		mtsp	<i>Metapolybia</i> sp.		X
rogr	<i>Ro. grandidieri</i> (de Saussure 1890)	X		mibas	<i>Mischocyttarus basimacula</i> (Cameron 1906)		X
rogu	<i>Ro. guttatipennis</i> (de Saussure 1853)	X		milbit	<i>Mischocyttarus labiatus</i> (Fabricius 1804)		X
roho	<i>Ro. horni</i> Sonan 1938	X		mimtt	<i>Mischocyttarus mattogrossoensis</i> Richards, 1978		X
sysu	<i>Synoeca surinama</i> (Linnaeus 1767)	XC	X	mimln	<i>Mischocyttarus melanarius</i> (Cameron 1906)		X
syvg	<i>Sy. virginea</i> (Fabricius 1804)	X		plinf	<i>Polistes infuscatus</i> Lepeletier 1836		X
				pllan	<i>Polistes lanio</i> (Fabricius 1775)		X

Figure 2). Most of the 127 polistine species are members of the tribes Mischocyttarini and Epiponini. Although a satisfactory sampling has been achieved for the Polistini

(with six of the eighth subgenera: *Polistes* s. str., *Polistella*, *Gyrostoma*, *Onerarius*, *Epicnemius*, *Aphanilopterus*), the 11 species of Ropalidiini represent a very small sample

FIGURE 2 Tree for studied vespids with branch lengths in a million-year scale; ages of labelled dated nodes in [Table S1](#) (partially based on fossils and molecular divergence). Remaining nodes adjusted with function “bladj” of Phylocom 4.2; species abbreviations as in [Table 1](#).



of the tribe's diversity. Nonetheless, all genera and many species groups of polistines are represented, being a comprehensive sample of the large body-size variation in the subfamily. We complemented the sample with species of the social subfamilies Vespinae and Stenogastrinae, as well as with representatives of the solitary Euparagiinae, Masarinae and Eumeninae, which differ in characteristics related to nesting materials and substrates and feeding habits.

The emphasis of the study is on interspecific variation, and because of the large number of species involved, we disregarded intraspecific variation using data from a single female specimen. However, to evaluate the magnitude of intraspecific variation, measurements of the femora and tibiae of the fore and hindlegs were made in individuals of 36 species of several polistine genera, using samples of 5 to 21 individuals (about 10 in most cases).

The total of 145 species of 41 genera from all six extant subfamilies of Vespidae were arranged in two datasets: D1 with 137 species, used in interspecific analyses, and D2 with 36 polistine species, used to assess the extent of intraspecific variation (Table 1).

2.2 | Observation and measurement

Dry-pinned specimens of each species were measured with an ocular micrometre, and voucher specimens are deposited in the insect collection of the Museu Goeldi. A ZEISS SV-11 microscope was used for collection of the interspecific data (dataset D1), and a MOTIC SMZ-140 microscope was used for intraspecific measurements (dataset D2).

Measurements of the femur, tibia and the whole complement of tarsal segments (tarsal total length) for one of each of the fore-, mid- and hindlegs (Figure 3a,b) were performed at the same magnification (2.0 \times), and the values in the original scale were used in subsequent analyses. In many specimens, the tarsi were bent or twisted, and the total length was approximated by the sum of partial

measurements of more or less straight stretches. The possible asymmetry between the right and left sides was disregarded (not noticeable anyway); thus, any of the members of a leg pair was measured after considering the specific conditions of a specimen's integrity and positional aspects of preservation.

The length measurements of the coxae were collected separately on part of the species of the interspecific dataset (D1 in Table 1), with this subsample having 36 species less than the complete dataset. However, inferences on ancestral values for the coxae morphometric variables were made on the complete tree of Figure 2, using weighted squared change parsimony (see below).

The formal analyses mainly used the length data of the tibiae and femora, which by their more exposed positions are easier to measure than the coxae. Tibiae and femora are also expected to have smaller measurement error (given their constitution as single rigid bars) than do the tarsi, which are composed of articulated segments often distorted in several ways.

2.3 | Size variable

We measured the height of the mesopleuron (from articulation of the midcoxa to the spiracular entrance, Mpl; Figure 3a,b) to control for size effects. Silveira and Silveira (1994) observed in a polistine interspecific dataset that this variable had a large correlation and was nearly isometric with body size (see also Silveira & Santos, 2011).

2.4 | Drawings and graphics

Drawings in Figure 3 were prepared by vectorization of photographs with the program Inkscape (<https://inksc ape.org>). Figures with phylogenetic trees were prepared with programs of the R Platform (see below), or with the program Mesquite 3.61 (Maddison & Maddison, 2019).

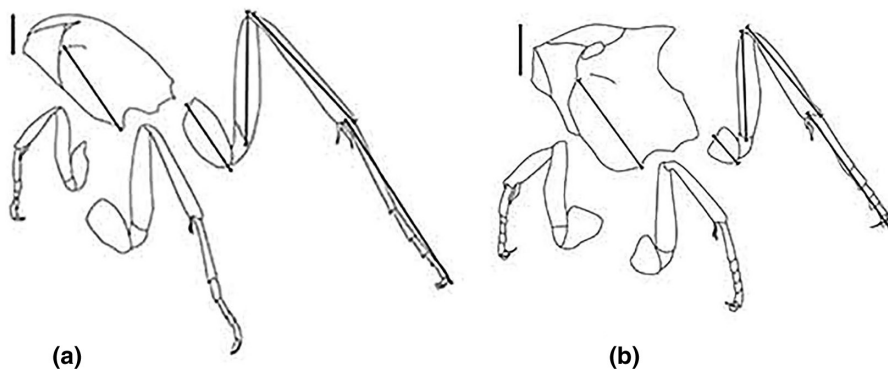


FIGURE 3 Schematic views of the mesosoma and legs in *Mischoctytarus* (a) and *Brachygastra* (b), with lines indicative of measurements (shown for mesopleuron and hind leg); vertical bar = 1 mm.

2.5 | Supplementary information

Supplementary tables and figures are available in the PDF file “Appendix S1.” Measurement data are available as trait/size ratios (trait length/Mpl) in spreadsheet format in the “Appendix S2.”

2.6 | Standard statistical analyses

Descriptive statistics and ordinary least squares regression (OLS) were computed using the program PAST (Hammer et al., 2001).

2.7 | Phylogeny-based analyses

Phylogenetic correlations were computed with Bayesian methodology, using BayesTraits v3.0.2; Meade and Pagel (2019). The significance of the coefficients relative to alternative zero correlation models was assessed with Bayes factor tests (Meade & Pagel, 2019; Oaks et al., 2018).

Phylogenetic regressions (Harmon, 2019; Revell & Harmon, 2022; Symonds & Blomberg, 2014) were performed with the functions “pgls” of the program caper 1.0.1 (Orme et al., 2018), and “gls” of the program ape 5.4–1 (Paradis, 2006)/nlme 3.1–151 (Pinheiro et al., 2020); both programs work in the R platform (RStudio 1.4.1717 © 2009–2021, PBC).

Maximum likelihood estimates of ancestral states of morphometric characters were computed using function “contMap” of the R program Phytools 0.7–70 (Revell, 2020).

All the methods above depend on a tree specifying the species phylogenetic relations, and whose branches have lengths proportional to some variation metric or to evolutionary time. In the present study, branch lengths are proportional to time in million years (see below). Trees are necessary because species are not statistically independent as instances of observation, so a phylogeny must be used to provide estimates of the species covariances. Evolution along the branches is often assumed to occur according to some stochastic model with a corresponding probability distribution. One such model commonly used in phylogenetic comparative studies of continuous characters is Brownian motion (BM) (Revell & Harmon, 2022; Schluter et al., 1997), and so is the case with the methods used here (Meade & Pagel, 2019; Orme et al., 2018; Pinheiro et al., 2020; Revell, 2020).

Weighted squared change parsimony (WSCP) was used for mapping of ancestral values for ratio variables

of the coxae, using the program Mesquite 3.61 (Maddison & Maddison, 2019). Because WSCP uses branch lengths to weigh the squared changes between tree nodes, the assigned values are numerically equivalent to maximum likelihood estimates under a BM evolution model (Webster & Purvis, 2002).

2.8 | Tree assembling

A phylogenetic tree for Vespidae was assembled with Mesquite 3.61 (Maddison & Maddison, 2019), including the sampled 119 species of all polistine genera plus 18 species of other vespidae subfamilies. Relationships at the subfamily level were represented as in Carpenter (1982) and Perrard et al. (2017), with Stenogastrinae as a sister group to (Vespininae + Polistinae), and with a monophyletic Eumeninae. In the second paper, a close relationship was suggested between Zethini and Odynerini, but only three eumenines were used in that dataset designed to specify higher-level relations in a study of fossil vespids. Therefore, regarding the relationships of the Eumeninae species, we referred to Hermes et al. (2014), who presented Zethini as a basal lineage and sister group to other Eumeninae subgroups.

Relationships between Euparagiinae and Masarinae were also specified according to Carpenter (1982) and Perrard et al. (2017), with a monophyletic Masarinae (contrary to Piekarski et al., 2018). The relationships among the three sampled Vespininae were constructed as in Perrard et al. (2015), with *Vespa* and *Provespa* as sister groups and with a paraphyletic *Vespula* (*Dolichovespula* as sister group to other vespines).

Regarding the polistine tribes, the figured relations are in accordance with Pickett and Carpenter (2010), Piekarski et al. (2018) and Menezes et al. (2020), with Polistini as a sister group to the Epiponini. The Ropalidiini, in general, were construed as in Carpenter (1993), with the gross taxonomic structure in *Ropalidia* as suggested in the recent literature, and based on nest architecture and behaviour (Kojima, 1982, 1996; Richards, 1978b; Vecht, 1941). The Mischocyttarini were represented as in Silveira (2008), and the Polistini according to Somavilla et al. (2021). The Epiponini, in general, were figured as in Menezes et al. (2020), with some relations in *Agelaisia* following the arrangement of species groups in Richards (1978), and other cases as suggested in a paper by Silveira and Carpenter (1995). *Pseudopolybia* was treated as in Andena et al. (2007), and *Protopolybia* as in Santos et al. (2015). Some lower-level resolution decisions were taken in the genera *Parachartergus*, *Metapolybia* and *Clypearia* in accordance with the arrangements in Richards (1978).

2.9 | Tree branch lengths (dating)

Tree node dating was performed by initially using age estimates from the literature for 33 divergence events (Table S1), and then using the function “bladj” in the program Phylocom 4.2 to adjust the height of the remaining 78 nodes by placing them evenly between dated nodes, and between dated nodes and terminals. This adjustment minimizes the variation in the length of the branches within the restrictions imposed by the dated (fixed) nodes (Webb et al., 2011; Phylocom manual).

The root of the tree was dated according to Perrard et al. (2017; a minimum age estimate for basal divergence of major vespidae lineages, based on fossils). The ages of three divergence events in *Mischocyttarus* (i.e., divergence of the genus crown-group, of subgenus *Mischocyttarus* s. str. and subgenus *Kappa*) were taken from work in progress (Dr. thesis by S. Felizardo, 2019) based on molecular data. Other estimates for 27 divergence events in Vespidae (mostly in Epiponini) were extracted from Menezes et al. (2020; based on molecular divergence). Because these authors used a relatively small sample of the taxonomic diversity of genus *Polistes*, resulting in a possibly too late divergence (ca. 20mya), we preferred not to fix the age of this genus/tribe, allowing it to be adjusted by “bladj.”

2.10 | Working tree

Figure 2 shows a phylogenetic tree for the studied Vespidae resulting from the assembly and dating methods described above. This should not be considered as a super tree in the narrow sense, because it results mainly from the pasting of non-overlapping partial phylogenetic hypotheses with different taxonomic scopes, and no special technique was necessary for solving conflicts.

Although several molecular studies have indicated a basal position for the Stenogastrinae in Vespidae (Hines et al., 2007; Piekarski et al., 2018; Schmitz & Moritz, 1998), we used the hypothesis of a single origin of eusociality in the clade (Stenogastrinae (Vespinae + Polistinae)) (Carpenter, 1982; Perrard et al., 2017; Pickett & Carpenter, 2010) because pure molecular-based studies still lack more complete sampling in all the subfamilies, especially those with solitary species (see Silveira et al., 2021). Additionally, the phylogenomic paper of Piekarski et al. (2018) did not provide times of divergence between the lineages, and there is no specific age estimate available for a putative basal split between the Stenogastrinae and its sister lineage encompassing the remaining Vespidae.

2.11 | Reduced (pruned) tree for Polistinae with all the nodes having associated “true” estimates of dates

Alternative regression analyses (OLS and PGLS) were performed with a reduced version of the polistine dataset, using a pruned tree with only 55 (or 46%) of the species used in the main analyses with the original Polistinae tree. This pruned tree (Figure S6) has all the internal nodes dated according to molecular analysis (Menezes et al., 2020). The purpose of this was to compare the results of regression analyses of the same variables in a subset of the taxa related according to a fully dated phylogenetic tree (a true chronogram), as opposed to a tree with part of the branch lengths specified after an arbitrary rule for height adjustment of internal nodes (a pseudo-chronogram partially constructed with the “bladj” method; see Molina-Venegas & Rodriguez, 2017).

3 | RESULTS

3.1 | Magnitude of intraspecific variation

Table 2 provides information on the intraspecific variation in 36 polistine species, given by estimates of the coefficient of variation (%). Measurements are for the fore femur (Fm1) and tibia (Tb1), hind femur (Fm3) and tibia (Tb3), and the size-variable height of the mesopleuron (Mpl). The coefficients of variation were generally low, approximately 5.0%. Additionally, there was good agreement between the values obtained for single individuals and the sample means for each of 28 species of the same group of species and genera (see Figure S7). Concordance between the two kinds of estimates is remarkable for most of the variables, either raw measurements or ratios. This combined evidence suggests that interspecific comparisons based on single-specimen data are fairly grounded, especially considering that our main interest is on large patterns of differentiation between major taxa.

3.2 | Size dependence (Polistinae)

In Vespidae, body shape varies greatly across genera and species, and leg-length proportions vary accordingly. In Figure 1, one can see a small species of *Mischocyttarus* (1A; wing-length ca. 7 mm) with proportionally very elongated legs. *Polistes* species (1B; wing-length ca. 20 mm) also normally have elongated legs, while in Epiponini there is much more variation, with *Agelais* and *Apoica* species

TABLE 2 Magnitude of intraspecific variation (as measured by the coefficient of variation, CV%) for the size variable (Mpl: height of mesopleuron) and four leg length variables (Fm1: forefemur, Tb1: foretibia, Fm3: hindfemur and Tb3: hindtibia) in 36 social species of 17 genera of Polistinae. (n: number of measured individuals); species abbreviations as in [Table 1](#).

Species	Mpl	Fm1	Tb1	Fm3	Tb3	(n)
agan	2.9	2.5	2.3	2.4	2.0	10
agfv	3.0	3.4	4.2	4.2	2.6	14
agpl	3.8	3.5	6.1	5.6	4.9	15
anpl	5.3	5.0	5.8	6.1	6.1	10
anpr	6.8	6.7	7.7	6.7	6.6	14
apflvs	3.1	2.9	7.9	3.3	1.9	9
astr	4.3	2.9	3.1	2.9	4.0	10
brlc	4.1	5.9	4.3	4.4	4.6	10
brsc	3.9	3.7	5.6	4.0	4.1	9
cgen	2.0	2.1	1.9	2.0	1.6	10
chmt	2.2	3.1	3.6	2.6	2.7	10
cysu	2.3	2.7	3.2	2.7	4.4	10
ledo	4.8	6.6	4.6	6.0	6.5	10
mibas	8.5	7.7	7.3	9.8	9.4	14
midcpt	7.8	8.4	12.1	8.6	8.5	12
midw	6.5	5.9	4.9	6.7	6.3	15
milab	4.9	5.0	7.7	6.6	7.4	15
mimln	5.9	5.0	6.9	7.5	8.3	12
mimtt	3.8	5.9	3.5	3.5	6.4	5
mirfd	9.0	8.7	9.8	10.2	10.7	19
mtsp	2.7	1.8	4.9	3.4	1.9	9
plcd	4.0	4.6	3.6	5.4	3.3	13
plinf	7.4	6.0	7.0	6.5	6.9	17
pllan	4.8	6.1	5.1	4.8	5.4	7
plsu	9.1	9.0	10.4	9.1	10.6	12
plvr	6.1	7.5	6.5	6.4	6.5	9
pobt	4.8	4.3	4.7	5.2	6.5	15
pocc	3.5	4.0	3.8	3.6	3.7	11
podi	3.4	4.2	2.3	3.2	2.9	9
porj	3.6	5.7	4.1	2.8	3.8	10
posr	3.9	4.6	4.9	4.7	3.0	21
ppch	4.3	5.0	5.3	4.6	4.7	11
ppex	3.2	2.9	3.5	2.7	2.3	10
prfr	5.1	5.7	7.1	4.5	5.9	10
psdff	1.7	2.5	2.8	3.3	3.6	12
sysu	3.3	3.1	2.7	3.0	3.2	15
<i>mean</i> (lower & upper values)	4.6 (1.7–9.1)	4.9 (1.8–9.0)	5.3 (1.9–12.1)	5.0 (2.0–10.7)	5.1 (1.6–10.7)	

having elongated legs, but with other genera such as *Brachygastra* (1F; wing-length ca. 7 mm) and *Protopolybia* (1G; wing-length ca. 5 mm) presenting stocky bodies and relatively very short legs. In between the extremes, there are several levels of intermediary shapes in genera such as *Polybia* ([Figure 1d,i](#)), *Synoeca* ([Figure 1e](#)), *Pseudopolybia*

([Figure 1h](#)) and others. So, leg shape variation is clearly phylogenetically structured but to approach the problem of spotting patterns in a phylogeny, we also need to deal in an appropriate way with the influences of size variation.

In the supplementary material ([Appendix S2 Raw Data](#)), we present values of length measurements for each

leg segment as ratios to mesopleuron height (trait/size ratios), with species ordered by size (Mpl) within each subfamily. In Polistinae, the smallest species tend to have the most different (and smallest) values. Another relevant aspect is that the greatest differences in proportions refer to parts of the mid and hindlegs, particularly the distalmost elements (i.e., tibia and tarsus).

Taking the hindtibia length as an example, if we compare the largest polistine species in our sample (*Polistes bicolor*) which is ca. 3.7 times larger (in linear size; mesopleuron height as proxy) than the smallest one (*Protopolybia holoxantha*), then the trait/size ratio for hindtibia in the large species is 1.34 while being only 1.08 in the smaller species. However, changes in proportion are not linearly gradual across the sample, and most differences in size-mediated shape of legs are between the smallest species and the other size classes. This pattern occurs generally for all the measured leg elements. So, strict geometric similarity between differently sized species does not hold in general, but differences are larger in comparisons involving very small species and the larger ones. Furthermore, our samples of the size classes are not free from phylogenetic bias, that is, there are no *Polistes* species with the size of *Protopolybia* species, and vice-versa (actually, some *Polistes*, e.g., in Old World subgenus *Polistella*, may indeed be considerably smaller than the here used species, but never as small as minute *Protopolybia*, or *Leipomeles* species).

Treating the size influence in a more formal way, Figure 4 shows the least square log–log regressions for the measurements of leg elements on the height of the mesopleuron (Mpl), both ordinary (OLS) and phylogenetic (PGLS) regressions. The importance of size is quite evident, and correlations are very high, slightly lower for mid and hind tibiae. Regressions for all the three tarsi follow closely the trends observed for the segmentally corresponding tibiae. Allometric slope coefficients (b) support the comment made before about size-related shape differences being larger in respect of mid and hind leg elements, and particularly for the distalmost ones (i.e., tibiae and tarsi). Values of “b” larger (or smaller) than 1.0, imply in expected shape differences along the size range, especially at the extremities.

OLS coefficients for the length of the tarsi are even higher than those for the tibiae, implying in that larger species tend to have longer mid and hindlegs. However, phylogenetic non-independence of observations evidently also applies here. Very large species in the sample are from only a few genera (mostly *Polistes*), and the same is true with respect to the lower end of the size range. To deal with this problem, we used the phylogenetic regression method making use of information for the Polistinae component in the tree of Figure 2. The two models of phylogenetic

regressions (BM: with fixed $\lambda = 1$; LAM: λ variable) produced allometric coefficients lower than the OLS regressions, and not all of them are significantly different from 1.0 (only coefficients for LAM are shown in Figure 4). In respect of the measurements for the mid and hindlegs, BM and LAM resulted very similar, with the respective regression lines almost indistinguishable visually. In the cases of foreleg elements, LAM resulted in between the other two models (OLS: no phylogenetic information; and BM: using unaltered tree branch lengths) in respect of line declivity.

The λ values for the LAM regressions resulted all lower than 1.0 meaning that the specified lengths of internal branches of the tree in Figure 2 (corresponding to estimates of species covariances) do not agree with the patterns of observed trait similarities between species as strongly as would be expected under a simple “constant rate” random model of character evolution (BM). Stronger disagreement was obtained in the cases of the foreleg elements (Figure 4; λ values: 0.71; 0.66; 0.67). The extra λ parameter of the LAM models tends to make them superior to both OLS and BM in explaining data variation (as confirmed by log-likelihood tests).

Allometric coefficients closer to 1.0 in the phylogenetic regressions contradict the instances of geometric dissimilarity as presented before through comparisons between species at the extremes of the size range and suggest that these should better be seen as sampling artefacts contingent on taxonomic composition.

When the phylogenetic issue was approached by simply making separate OLS regressions within each of the major polistine subclades, less steep allometric slopes resulted in most cases (Table 3), like those obtained in the phylogenetic regressions for the whole subfamily (Figure 4), with similarity being still more evident when comparison refers to the mean OLS slopes for each measured leg element across clades (Table 3).

The clade corresponding to the whole tribe Epiponini is omitted from Table 3 because it is already represented by smaller constituent subclades. Parameters for Ropalidiini (and Epiponini) are generally quite similar to those for Polistinae as a whole. This cannot be a surprise in the case of Epiponini since a majority of sampled species in almost every size class are members of this tribe. Body shapes of the ropalidiines, however, are poorly represented in our sample, especially of *Ropalidia* which is very diverse morphologically so that details of shape variation in this tribe cannot be adequately examined here. Except for these two tribes, all the regression lines for the remaining clades are less steep than the corresponding Polistinae line, with slopes closer to the isometry condition. Slopes for *Polistes* and the clade *Brachygastra-Protopolybia* are either very close to isometry or show negative allometry, but in the latter

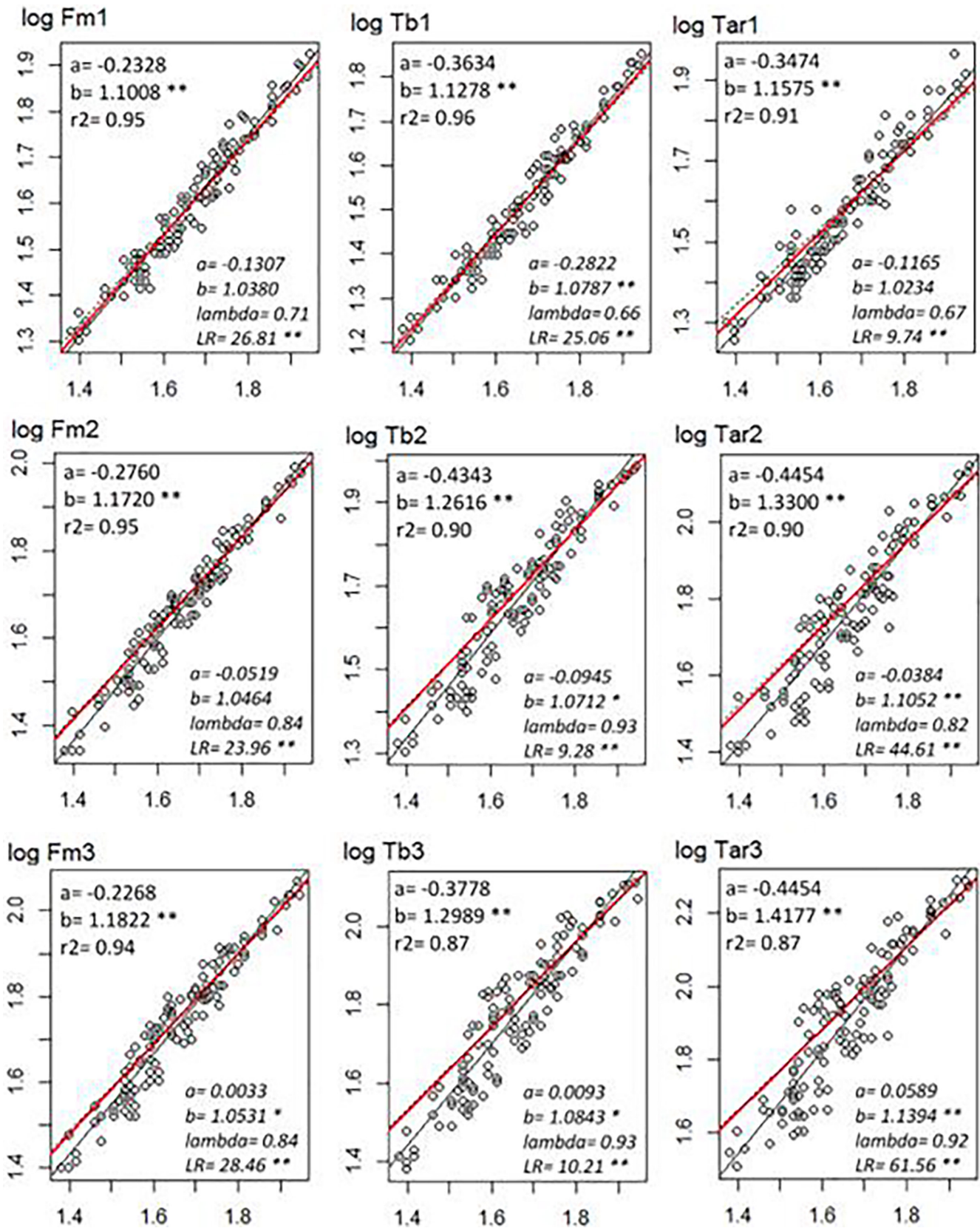


FIGURE 4 Regression log-log plots of leg-length variables (Fm1, Tb1, Tar1, Fm2, Tb2, Tar2, Fm3, Tb3 and Tar3) on size-variable height of mesopleuron (Mpl) for Polistinae. Coefficients on upper left from ordinary regression (OLS); on lower right from phylogenetic regressions (LAM model) based on polistine clade in Figure 2; a (intercept), b (regression slope), r² (coefficient of determination), lambda (Pagel's branch-length scaling parameter) and LR (likelihood ratio test relative to Brownian motion model with lambda = 1). Regression lines: OLS—continuous black; LAM model—continuous red; BM model—dashed black.

TABLE 3 Parameters and related statistics for OLS regressions within each major polistine clade, presented with the format: intercept-a + slope-b [standard error]; determination coefficient. FM: femur; TB: tibia; in parentheses after clade names is the number of sampled species.

		Foreleg	Midleg	Hindleg
Ropalidiini (11)	FM	-0.1544 + 1.0574 [0.0456]; 0.98	-0.3082 + 1.1881" [0.0547]; 0.98	-0.1842 + 1.1473 [0.0655]; 0.97
	TB	-0.3550 + 1.1309' [0.0409]; 0.99	-0.4373 + 1.2514" [0.0683]; 0.97	-0.3411 + 1.2689' [0.0991]; 0.95
Polistini (11)	FM	0.1633 + 0.8953 [0.1204]; 0.86	0.2439 + 0.8944 [0.1004]; 0.90	0.1937 + 0.9559 [0.1310]; 0.86
	TB	-0.1083 + 1.000 [0.1330]; 0.86	0.0972 + 0.9725 [0.1241]; 0.87	0.1673 + 0.9964 [0.1744]; 0.78
Mischocyttarini (24)	FM	-0.2552 + 1.0923 [0.0498]; 0.96	-0.0113 + 1.0307 [0.0517]; 0.95	0.0550 + 1.0430 [0.0456]; 0.96
	TB	-0.3498 + 1.1009 [0.0523]; 0.95	-0.0339 + 1.0627 [0.0732]; 0.91	0.1594 + 1.0301 [0.0739]; 0.90
Agel_Apoic (13)	FM	-0.1768 + 1.0841 [0.0524]; 0.98	-0.0549 + 1.0583 [0.0283]; 0.99	0.0578 + 1.0293 [0.0348]; 0.99
	TB	-0.4421 + 1.1819" [0.0517]; 0.98	-0.2102 + 1.1523" [0.0379]; 0.99	-0.1606 + 1.1928" [0.0598]; 0.97
Pseud_Parach (14)	FM	-0.0602 + 0.9882 [0.0496]; 0.97	-0.0893 + 1.0486 [0.0647]; 0.96	-0.0977 + 1.0878 [0.0576]; 0.97
	TB	-0.2264 + 1.0347 [0.0608]; 0.96	-0.1725 + 1.0745 [0.0677]; 0.95	-0.1805 + 1.1411 [0.0876]; 0.93
Astel_Polyb (28)	FM	-0.1765 + 1.0781 [0.0399]; 0.97	0.0146 + 0.9896 [0.0335]; 0.97	0.0518 + 1.0059 [0.0367]; 0.97
	TB	-0.3031 + 1.0994' [0.0434]; 0.96	-0.0822 + 1.0397 [0.0394]; 0.96	-0.0075 + 1.0635 [0.0592]; 0.92
Brach_Proto (16)	FM	0.1633 + 0.8318 [0.0830]; 0.88	0.0528 + 0.9266 [0.0803]; 0.90	0.0705 + 0.9557 [0.0695]; 0.93
	TB	0.0652 + 0.8438 [0.0914]; 0.86	0.1307 + 0.8479 [0.0762]; 0.90	0.0971 + 0.9414 [0.0684]; 0.93
mean b (cv %)	FM	1.0039 (10.3)	1.0195 (9.5)	1.0321 (6.7)
	TB	1.0559 (10.5)	1.0573 (12.1)	1.0906 (10.6)
mean a (sd)	FM	-0.0709 (0.1698)	-0.0218 (0.1660)	0.0210 (0.1238)
	TB	-0.2456 (0.1735)	-0.1012 (0.1949)	-0.0380 (0.1947)

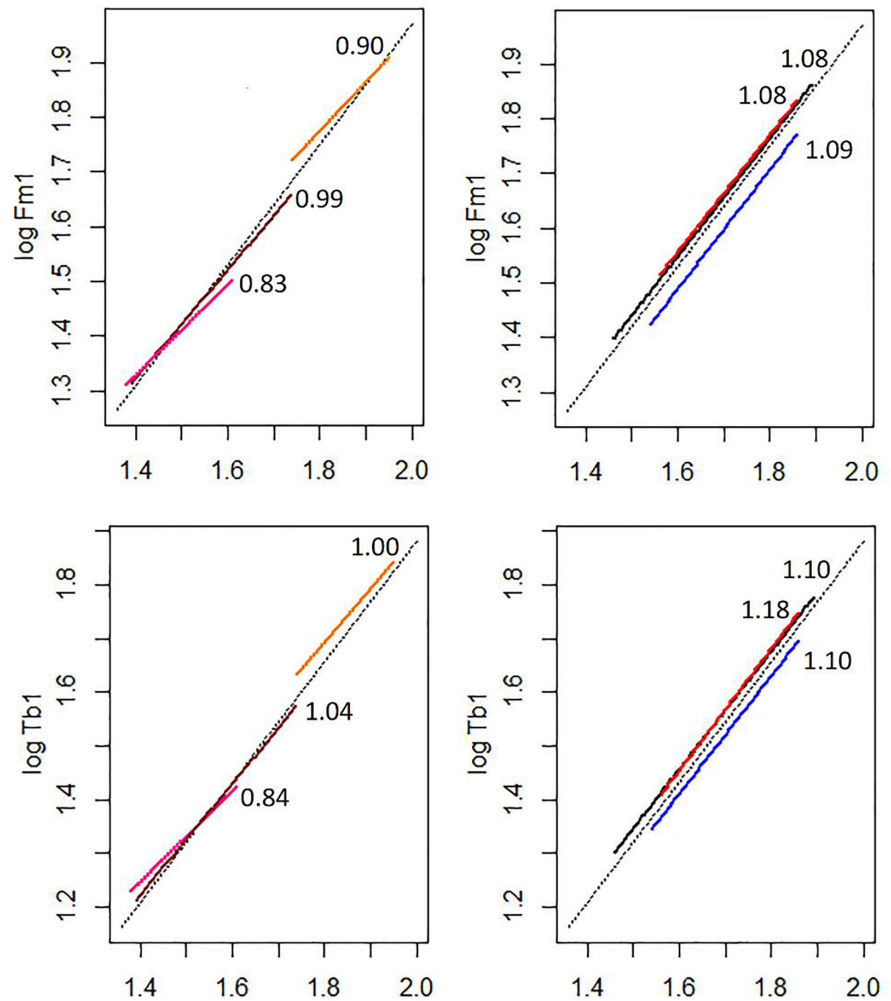
case, the differences relative to the 1.0 reference value are not statistically significant.

In accordance with the nearly isometric relations in subclade regressions, direct comparisons of leg-length proportions within the clades reveal smaller differences between species on the extremes of the size range in most cases (except for Ropalidiini and Epiponini as a whole), even when the size difference is still considerable. For example, within *Mischocyttarus*, the size ratio for hindtibia in the small species "*M. sp. nr. prominulus*" (WL ca. 7 mm) is 1.51 and in the much larger *M. flavicans* (WL ca. 16 mm) it is 1.60. In the epiponine clade *Asteloeca-Polybia*, this same ratio is 1.28 in *Metapolybia suffusa* (WL ca. 7 mm) and 1.32 in *Synoeca surinama* (WL ca. 20 mm).

In **Figures 5** and **6**, one sees log-log graphics with OLS regression lines for respectively the fore and hind femora and tibiae (differently coloured for each polistine subclade), which are presented in groups of three. A reference black-dashed line represents the regression for the whole subfamily Polistinae; regression lines for Ropalidiini and Epiponini are omitted.

An important aspect of these graphics (**Figures 5** and **6**) refers to differences in regression intercepts (see also **Table 3**), and how they relate to clear differences in the shape of the legs between clades that are independent of size. For example, for the mid and hindlegs (**Figure 6**), the *Mischocyttarus* lines (in blue) are always above those of the other clades, meaning that *Mischocyttarus* species have longer mid and hindlegs than species of other

FIGURE 5 Log–log graphs with OLS regression lines for the fore femur (log Fm1) and tibia (log Tb1) for polistine subclades: *Polistes* (dark orange), *Pseudopolybia-Parachartergus* (dark brown), *Brachygastra-Protopolybia* (deep pink), *Agelaia-Apoica* (red), *Asteloeca-Polybia* (black) and *Mischocyttarus* (blue). X-axis refers to mesopleuron height (Mpl); general all-Polistinae regression represented by a dashed black line; associated numbers are regression slopes.



genera independently of body size. This is reversed when considering the forelegs (Figure 5), for which *Mischocyttarus* regression lines are below those of other clades. Contrary to slope coefficients, intercept values varied greatly between clades (see Table 3 for mean values and dispersion measures for these coefficients across clades). Intercept values (and slopes) per leg element were not significantly correlated to mean body size across clades, which supports the conclusion about size-independence of shape aspects informed by the intercept differences. Furthermore, these same aspects are also reflected on the residuals of the main analyses for the whole Polistinae.

The graphs in Figures 7–10 show the relationship between the distributions of OLS residuals and phylogeny; each figure presents the vespid tree, and a bar diagram—to the right—representing the residuals for the length of the fore and hind femora and tibiae (results for the midleg elements follow closely those for hind-leg), for the Polistinae only. One can easily see that the residuals are structured on a phylogenetic basis. These same Figures 7–10 also show maximum likelihood estimates of ancestral values of the ratios of each of these

leg elements relative to the size-variable height of mesopleuron (computed with R package phytools 0.7–80). Ratio values are represented on the branches of the tree through a continuously grading colour scale. There is a close agreement between the distributions of these trait/size ratios and the corresponding regression residuals across the tree. Because both the residuals and ratios are based on the same size variable, such an agreement is in a sense trivial. However, this indicates that these (computationally simpler) ratios might be an option as shape variables, which in great measure represent size-independent aspects of shape (although not strictly size-free; see below).

In Table 4, correlations are presented between each of the ratios for the various leg elements and the size variable (Mpl) for Polistinae. When measured by the conventional Pearson method, all ratios showed coefficients close to 0.5 (COLUMN 1 of matrix), which cannot be exactly considered a high level of correlation for such a relatively large sample ($n = 119$). Figure S8 shows the corresponding scatterplots which also help assess the relative weakness of these correlations (which are still lower if non-polistine vespids are included).

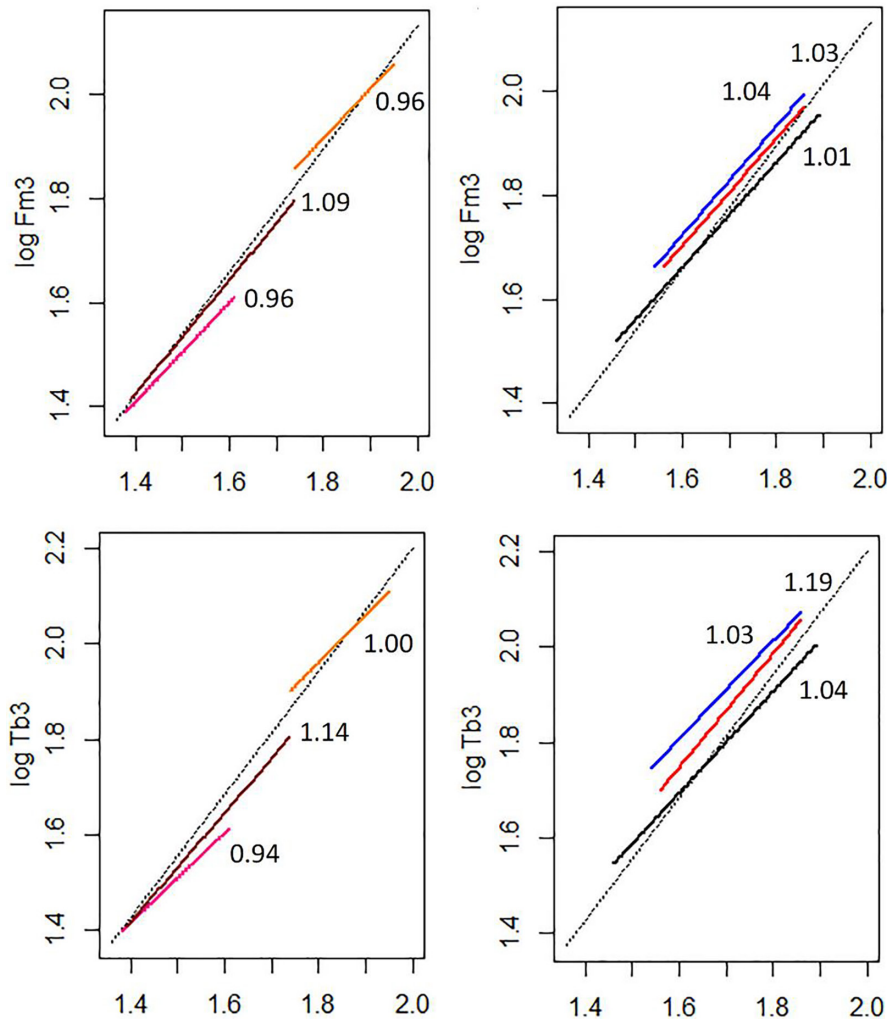


FIGURE 6 Log–log graphs with OLS regression lines for the hind femur (log Fm3) and tibia (log Tb3) for polistine subclades: *Polistes* (dark orange), *Pseudopolybia-Parachartergus* (dark brown), *Brachygastra-Protopolybia* (deep pink), *Agelaia-Apoica* (red), *Asteloeca-Polybia* (black) and *Mischocyttarus* (blue). X-axis refers to mesopleuron height (Mpl); general all-Polistinae regression represented by a dashed black line; associated numbers are regression slopes.

Furthermore, when correlations were computed taking into account the phylogenetic relationships between the species (with BayesTraits V3.0.2; ROW 1 of the matrix in Table 4), the estimated coefficients are all very low (below 0.2), and with respect to significance, they are scored at most as “positive evidence” based on Bayes factor tests.

By contrast, correlations between the trait/size ratios and the OLS residuals were all very high, regardless of the method used (Pearson or phylogenetic correlation; Table 4, last two COLUMNS at the right of the matrix), with such strong relations already apparent in Figures 7–10.

3.3 | Shape revealed by trait/size ratios

Given that trait/size ratios had low correlations with the mesopleuron size variable and high correlations with regression residuals, we use them to compare leg shape among vespine species. Because of ratios' intuitive appeal and simplicity of computation (compared with residuals),

they also make it easier to refer to the non-polistine species excluded from formal regression analyses for reasons of avoiding too much taxonomic/behavioural heterogeneity, or simply because sampled species do not sufficiently represent a given major taxon (e.g., the Eumeninae, the largest subfamily in Vespidae with 210 genera and more than 3500 species are obviously underrepresented in this study).

Taking again the tibiae as examples, in Figure 8 the estimated ancestral values for the ratio-variable rTb1 (length of foretibia/height of mesopleuron) indicate how changes in this proportional relationship may have occurred in Vespidae. Intermediary values (ca. 0.72; green colour) were inferred at the base of the Polistinae clade, and mostly similar values resulted at several successive internal nodes and in most of the Epiponini lineages. Trends for proportionally shorter foretibiae were the most remarkable in *Mischocyttarus* (values down to 0.59), in the clade encompassing *Pseudopolybia* and *Parachartergus*, in some members of *Polybia*, and in the clade *Brachygastra-Protopolybia*. Trends for proportionally longer foretibiae were indicated mainly in *Belonogaster*, in most *Polistes*, in *Apoica*, less strongly in a few elements of *Polybia*,

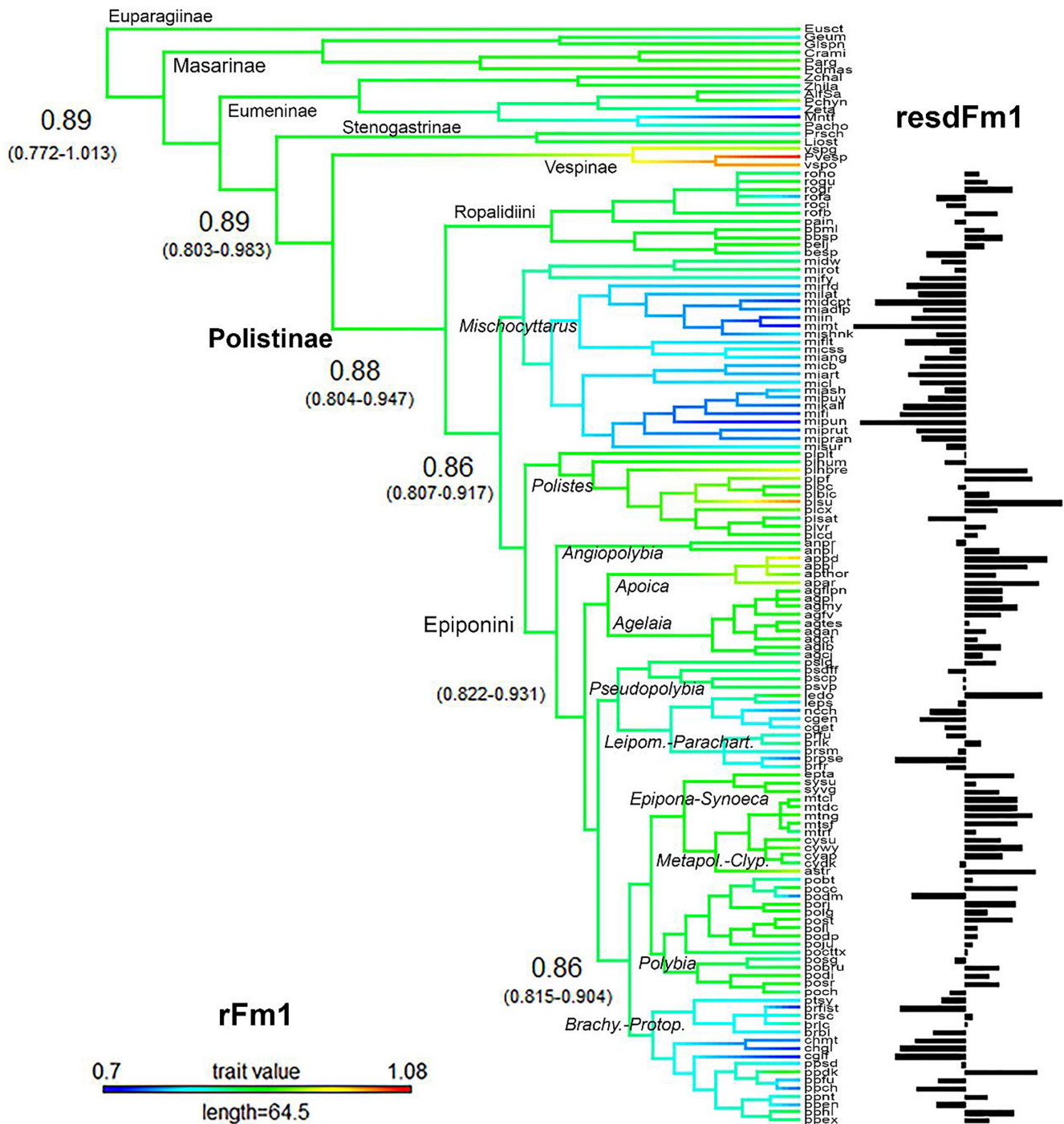


FIGURE 7 “Tree-with-bars” showing (left) max. likelihood estimates of ancestral states for ratio $rFm1$ (length of forefemur/height of mesopleuron) on tree of Figure 2 (with R package phytools 0.7–80); values presented for some nodes with 95% confidence limits. Bars (right) refer to ordinary OLS residuals ($rsdFm1$) of log–log regression of $Fm1$ on Mpl , positive values to the right; species abbreviations as in Table 1.

and in members of the *Synoeca-Clypearia* clade. Outside Polistinae, relatively longer foretibiae, were observed mainly in the vespine species.

In Figure 10, an analogous portrait of inferred changes is presented for the ratio-variable $rTb3$ (between the length of the hindtibia and height of the mesopleuron) in Vespidae. The picture shows a general

trend of secondary shortening of the hind tibia in Epiponini, with the accentuation of this in the clades *Pseudopolybia-Parachartergus*, and *Brachygastra-Protopolybia*. A similar independent shortening trend was inferred in part of the Ropalidiini in the genus *Ropalidia*. An opposite lengthening trend for the hind tibia (actually to both mid and hind legs) is inferred in

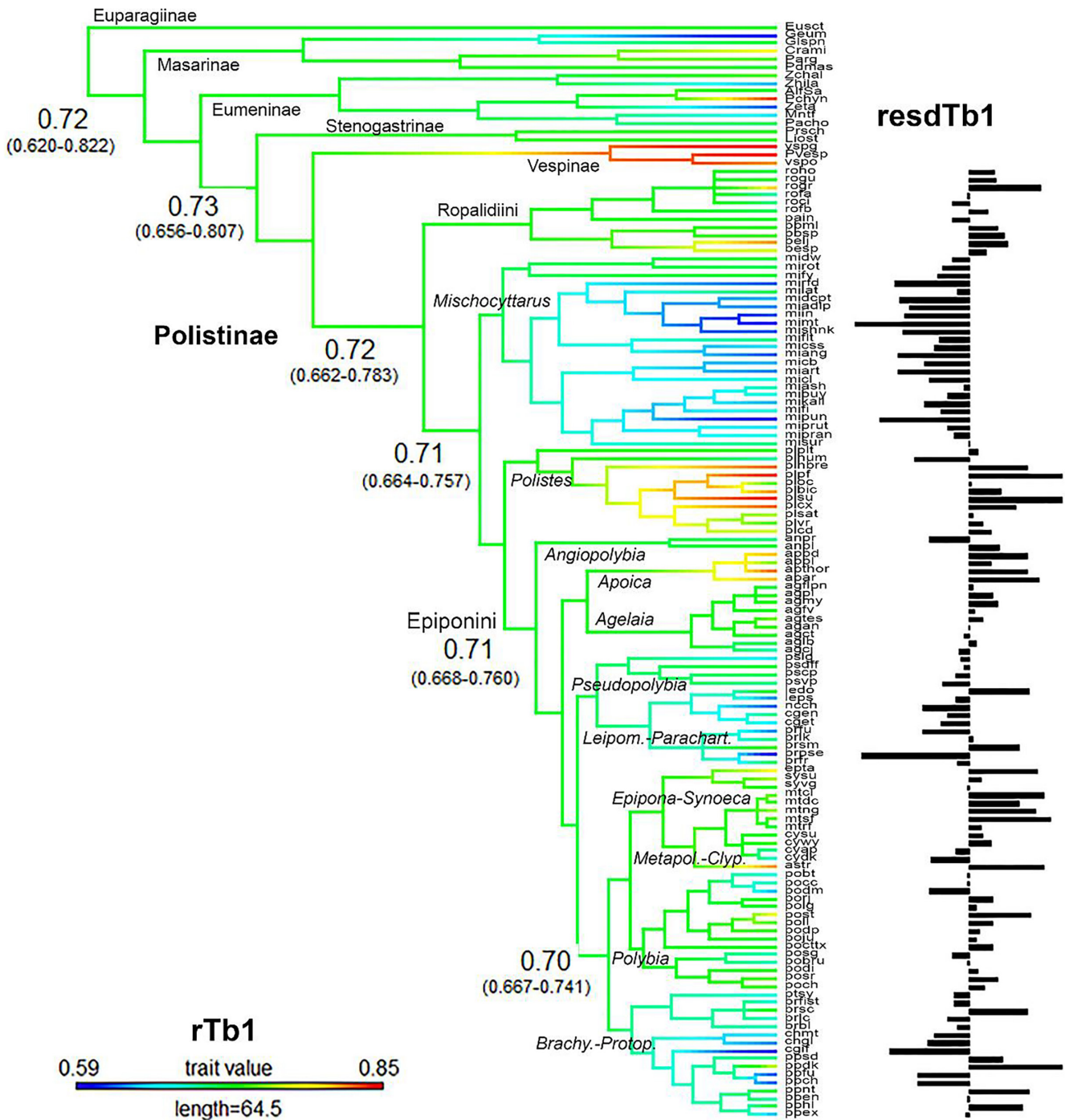


FIGURE 8 “Tree-with-bars” showing (left) max. likelihood estimates of ancestral states for ratio $rTb1$ (length of foretibia/height of mesopleuron) on tree of Figure 2 (with R package phytools 0.7–80); values presented for some nodes with 95% confidence limits. Bars (right) refer to ordinary OLS residuals ($rsdTb1$) of log–log regression of $Tb1$ on Mpl , positive values to the right; species abbreviations as in Table 1.

Mischocyttarus, and to some extent also in *Polistes* and *Apoica*. In *Mischocyttarus*, however, the lengthening of the hindlegs is generally much more pronounced (values of $rTb3$ may reach up to 1.81). Patterns of length changes for the midtibia ($rTb2$) were very similar to those for the hind tibia ($rTb3$), and patterns for each of the femora generally agreed well with that of each counterpart tibia.

Optimization of these ratio variables on the tree by the linear parsimony method (with program TNT v. 1.5, 2021; Goloboff & Morales, 2023) was also tried and the resulting patterns were remarkably similar to those obtained with maximum likelihood (see Figures S9–S14). One important difference in the linear parsimony method is that more often a range of values is assigned to ancestral nodes, instead of a single value.

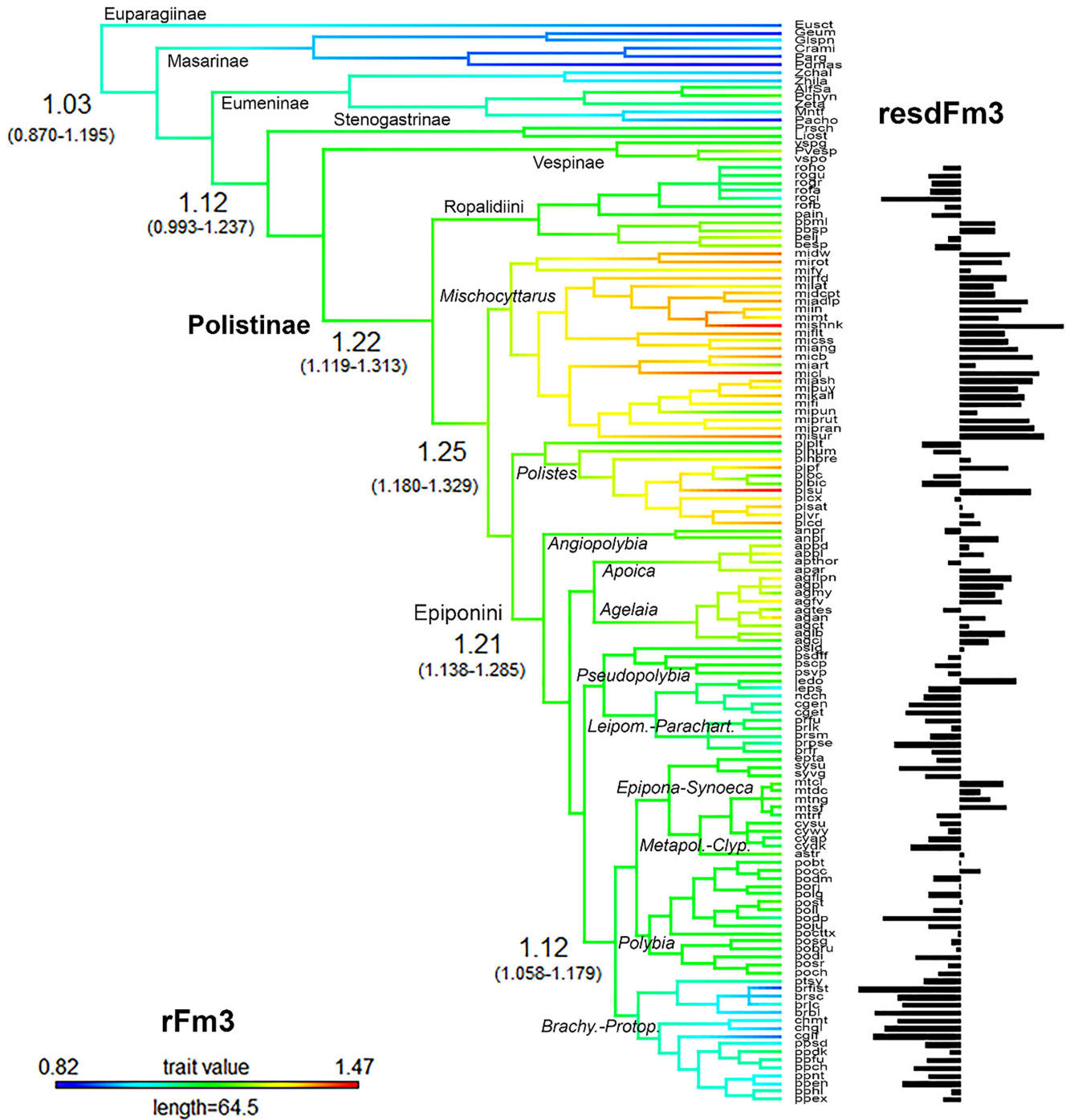


FIGURE 9 “Tree-with-bars” showing (left) max. likelihood estimates of ancestral states for ratio rFm3 (length of hindfemur/height of mesopleuron) on tree of Figure 2 (with R package phytools 0.7–80); values presented for some nodes with 95% confidence limits. Bars (right) refer to ordinary OLS residuals (resdFm3) of log–log regression of Fm3 on Mpl, positive values to the right; species abbreviations as in Table 1.

3.3.1 | Evolutionary trajectories

The pictures for the trait/size ratio variables presented generally two main kinds of evolutionary trajectories. In the case of the fore femur and tibia, the estimated ancestral values for some selected deep nodes across the tree were quite similar, that is, referring to ancestors of (1) Masarinae + (Eumeninae + eusocial vespids), (2)

eusocial vespids, (3) Polistinae, (4) Mischocyttarini + (Polistini + Epiponini), (5) Epiponini and (6) the *Epipona-Protopolybia* clade (Figures 8 and 9).

In the cases of the mid and hind femora and tibiae, however, the estimated values tended to be lower at the base of the tree, then increased with the origin of eusocial vespids and finally decreased again within the more advanced Epiponini (Figures 9 and 10).

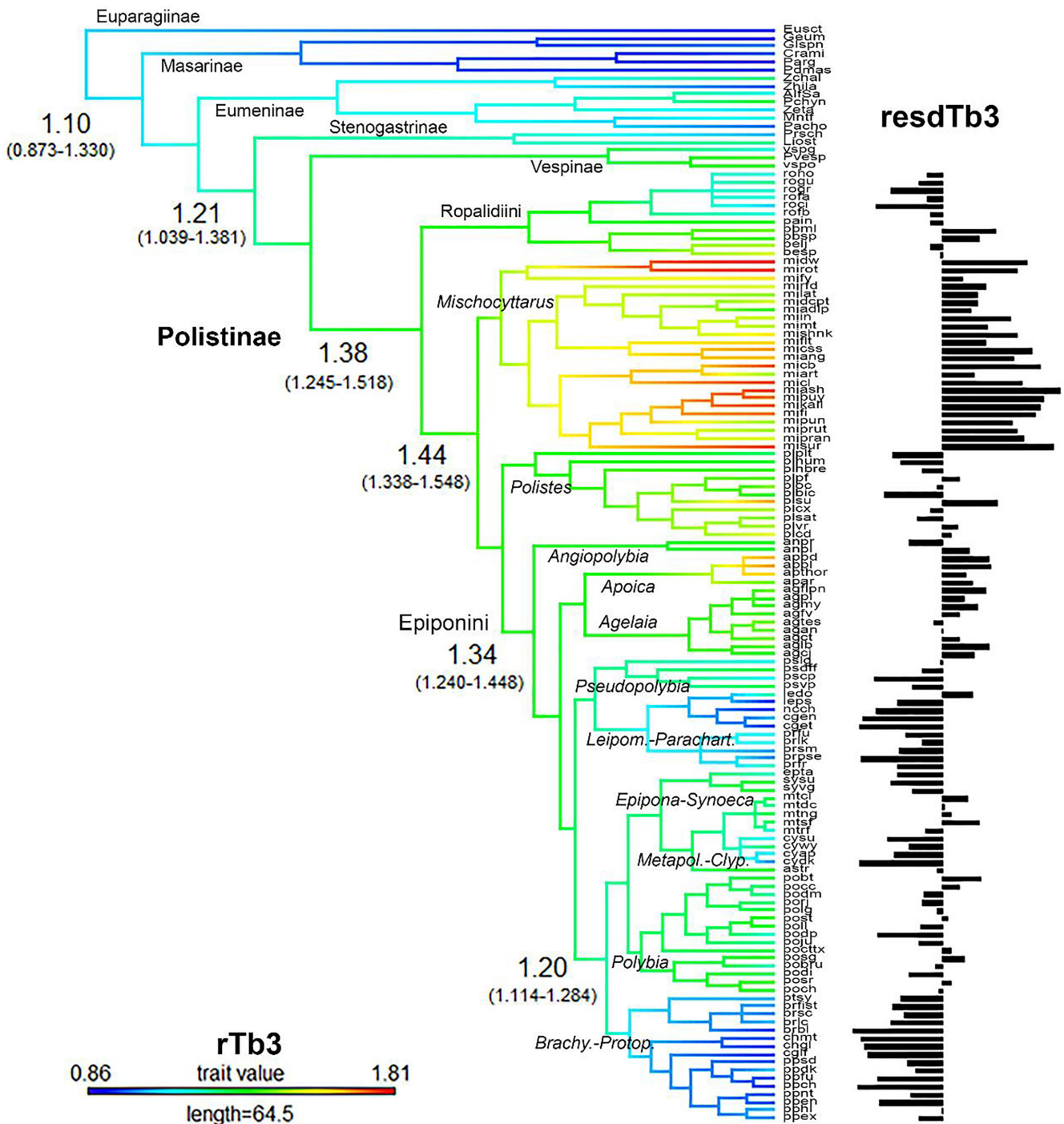


FIGURE 10 “Tree-with-bars” showing (left) max. likelihood estimates of ancestral states for ratio $rTb3$ (length of hindtibia/height of mesopleuron) on tree of Figure 2 (with R package phytools 0.7–80); values presented for some nodes with 95% confidence limits. Bars (right) refer to ordinary OLS residuals ($resdTb3$) of log-log regression of $Tb3$ on Mpl , positive values to the right; species abbreviations as in Table 1.

3.3.2 | The tarsi

The variation in the trait/size ratios for each of the tarsi ($rTars1$, $rTars2$ and $rTars3$; Figures S3–S5), was generally congruent with that of the other corresponding leg elements, especially for the mid and hindlegs. However, some discrepancies were observed regarding the

foretarsus ($rTars1$, Figure S3) in solitary subfamilies and in *Mischocyttarus*. Trends for the relative tarsal lengths in these taxa differ from those for the respective fore femur and tibia (and in *Mischocyttarus*, the changes in tarsal length are in the opposite direction, that is, of a proportionately longer foretarsus for species of the genus) (compare Figures 8 and 9 and Figure S3).

TABLE 4 Matrix of correlations among size variable (Mpl) and trait/size ratios for legs (computed by dividing each leg measurement by Mpl), for polistine species only.

	Mpl	rFm1	rTb1	rFm2	rTb2	rFm3	rTb3		
Mpl	–	0.004 ^w	0.18 ^p	0.06 ^w	0.16 ^p	0.08 ^w	0.15 ^p	<i>OLS residuals</i>	<i>Phylo. adj. residuals</i>
rFm1	0.40	–	0.74 ^{vs}	0.65 ^{vs}	0.61 ^{vs}	0.65 ^{vs}	0.45 ^{vs}	rsdFm1	0.92
rTb1	0.52	0.87	–	0.57 ^{vs}	0.54 ^{vs}	0.56 ^{vs}	0.46 ^{vs}	rsdTb1	0.87
rFm2	0.52	0.40	0.40	–	0.76 ^{vs}	0.82 ^{vs}	0.68 ^{vs}	rsdFm2	0.84
rTb2	0.49	0.22	0.25	0.91	–	0.80 ^{vs}	0.88 ^{vs}	rsdTb2	0.85
rFm3	0.47	0.23	0.24	0.93	0.94	–	0.77 ^{vs}	rsdFm3	0.86
rTb3	0.46	0.12 ^{ns}	0.18 ^{ns}	0.85	0.97	0.92	–	rsdTb3	0.86

Note: Above the main diagonal are phylogenetic correlations calculated with BayesTraits V3.0.2 (Meade & Pagel, 2019) using polistine clade in Figure 1. Below the diagonal are Pearson correlations. On the right-side end columns are correlations between ratios and residuals of log–log regressions of leg variables on Mpl, both OLS and phylogenetically adjusted residuals (for the latter using BayesTraits). Superscript symbols associated with coefficients: ns (non-significant; $p = .05$); w (weak evidence), p (positive evidence), vs (very strong evidence); last three according to values of “Log BF” (Bayes Factors; see Meade & Pagel, 2019).

3.3.3 | Integrated sets of appendages

Correlations among the ratio variables (for all femora and tibiae) are presented on Table 4, both the Pearson (below diagonal) and phylogenetic correlations (above diagonal), for Polistinae.

The coefficients have larger values when the measured association involves exclusively elements of the foreleg, or else elements of the mid and hindlegs, suggesting a system composed of two differently integrated sets of appendages. When measured by the phylogenetic method, the differences between the two groups of correlation coefficients were smaller but still noticeable.

Table 5 shows correlation coefficients among the OLS residuals of the size regressions, again for the Polistinae only. As in Table 4, coefficients measuring association “within” the fore or mid/hind leg groups are larger than coefficients involving leg elements “across” the two groups.

Finally, a similar pattern of correlation differences is apparent in the loading coefficients of the second principal component (PC2) of a principal component analysis for the six log-transformed femora and tibia measurements (Table 6; Polistinae only), with coefficients referring to lengths of the fore femur and tibia being large and positive, and those for the mid and hind leg elements being either near zero (mid femur) or negative for the remaining elements. PC1 was a typical size component, and the distribution of variances between the two components was also typical (PC1: 96.9% and PC2: 2.6%; see Table 6 for the remaining components).

3.4 | Shape variation of the coxae

Measurement data describing the proportional relations of the lengths of the fore, mid and hind coxae

were analysed as two inter-leg ratios (rCx1_Cx3 and rCx1_Cx2). These two ratio variables were optimized on the Vespidae tree by WSCP (with Mesquite 3.61), and the corresponding graphics are presented in a mirrored fashion in Figure 11. Not all species were measured, but the unmeasured terminals were maintained in the trees so that the relative importance of the corresponding information gaps can be appreciated.

The relations between the lengths of the coxae follow the main trends observed for the other leg segments. In *Mischocyttarus*, the forecoxa is proportionally much shorter than the mid and hind coxae, in close agreement with the patterns obtained for the femora and tibiae and differs from that of most other vespidae taxa.

By contrast, in most basal vespidae lineages, the forecoxa tends to be distinctly longer than the mid and hind coxae. In most of the Polistini and Epiponini, these relations are close to equivalence, except for the cases of extremely short-legged species, such as those in the *Brachygastra-Protopolybia* clade, which present proportions similar to those observed in solitary vespidae.

In summary, long-legged polistine species such as those in *Mischocyttarus*, *Polistes*, *Apoica* and *Agelaia* tend to have distinctly longer (and bulky) mid and hind coxae, while most epiponines tend to have all three serial coxae subequal in length; or in members of the genera *Brachygastra* and *Protopolybia*, the forecoxa may be comparatively longer.

4 | DISCUSSION

4.1 | General patterns of proportions

The indications by Duncan (1939) for the proportional relations between legs and leg parts of *Vespula pensylvanica*

	rsdfm1	rsdtb1	rsdfm2	rsdtb2	rsdfm3	rsdtb3
rsdfm1	–	0.75	0.66	0.64	0.62	0.48
rsdtb1	0.85	–	0.59	0.58	0.53	0.50
rsdfm2	0.26	0.18	–	0.74	0.80	0.65
rsdtb2	0.06 ^{ns}	0.02 ^{ns}	0.88	–	0.77	0.87
rsdfm3	0.07 ^{ns}	0.01 ^{ns}	0.91	0.92	–	0.78
rsdtb3	–0.05 ^{ns}	–0.04 ^{ns}	0.81	0.96	0.91	–

Note: Above main diagonal (italics) are phylogenetic correlation coefficients (BayesTraits V3.0.2; Meade & Pagel, 2019) for phylogenetically adjusted residuals calculated using the polistine clade in Figure 1. Below diagonal are Pearson coefficients for OLS residuals. Superscript symbols: ns (non-significant; $p = .05$); all phylogenetic correlations have “very strong” supporting evidence according to values of “Log BF” (Bayes Factors).

TABLE 6 Principal components analysis on the covariance matrix of six log-transformed leg variables (femora and tibiae), for Polistinae only, with loadings and eigenvalues (in percentage of total variance) computed with Past 2.17. Shaded cells mark coefficients for foreleg measurements on PC2.

	PC1	PC2	PC3	PC4	PC5	PC6
LFm1	0.365	0.565	–0.022	–0.518	0.528	–0.027
LTb1	0.373	0.563	–0.384	0.463	–0.417	0.092
LFm2	0.400	0.013	0.583	0.036	–0.264	–0.655
LTb2	0.440	–0.311	–0.068	–0.558	–0.503	0.375
LFm3	0.407	–0.140	0.472	0.419	0.328	0.556
LTb3	0.457	–0.497	–0.535	0.168	0.343	–0.335
% variance	96.9	2.6	0.2	0.1	0.1	0.04

correspond well to the most common patterns found in the presently studied vespids. The first is that the hindleg parts are longer than serially homologous midleg parts, which in turn are longer than the corresponding foreleg parts.

However, exceptions occurred regarding elements of fore and mid legs (in ca. 10% of the studied species), in which the homologue elements are practically of the same length, or even with the relation being inverted so that the foreleg elements are slightly longer. This was found principally in some epiponine species with stout body and very short legs (in *Brachygastra* and *Protopolybia*), and in some solitary species of Euparagiinae, Masarinae and of the eumenine genera *Zethus* and *Pachodynerus*. We suspect that in the social groups this reflects a general trend of shortening of the legs, while at least in some of the solitary species, it may be more the case that forelegs are larger than usual, perhaps because of excavating functions.

Regarding the proportions between complementary parts within each leg, foretibia was always distinctly shorter than forefemur in all vespids as reported by Duncan (1939) for *V. pennsylvanica*, while for the mid and hind legs, the relation varied to some extent.

TABLE 5 Matrix of correlations among residuals of log–log regressions of six leg variables on the size variable (Mpl) for Polistinae only. Shaded cells mark coefficients for relations involving fore X mid-or-hind legs.

Midtibia was shorter than midfemur (as pointed by Duncan, 1939) in ca. 40% of the species, or about the same length as midfemur in other 40% of species, and clearly longer than midfemur in nearly 20% of the species (mostly *Mischocyttarus* and *Apoica*). Such variation was correlated to general elongation of the legs as measured by the trait/size ratio rTb3 (Figure 10). Regarding the hindleg, 64% of the species had the tibia clearly longer than femur, and most of the remaining had them with about the same length, with only five species having a shorter tibia, typically stout-bodied species of the masarine genus *Paragia*, and of the social *Nectarinella*, *Chartergellus* and *Protopolybia*.

4.2 | Legs' biological roles and morphometric variation

Textbooks on Vespidae usually treat the legs of these wasps as morphologically “unspecialized” due to the variety of tasks they must perform (Duncan, 1939; Edwards, 1980; Spradbery, 1973). Table 7 shows a list of uses of the legs by vespid wasps, based mainly on the published information. Most of these elementary actions or behaviours have general character and distribution. However, some are expected to be performed mainly by certain groups, for example, item 8 is related to the ability to forage on spider webs and has been reported in species of *Mischocyttarus* and Stenogastrinae, or the postural behaviour in item 11 that has been observed only in some *Mischocyttarus* (see Figure 1c). Item 14 (substrate excavation) is typical of digger solitary vespids, and item 16 is related to the provisioning behaviour of solitary eumenines. Items 7 (hanging from tarsal claws) and 10 (raising head and forelegs) are probably mainly performed by eusocial species.

A relevant point emerging from the inspection of some items in Table 7 refers to the considerable independence of the forelegs in relation to the mid and hindlegs,

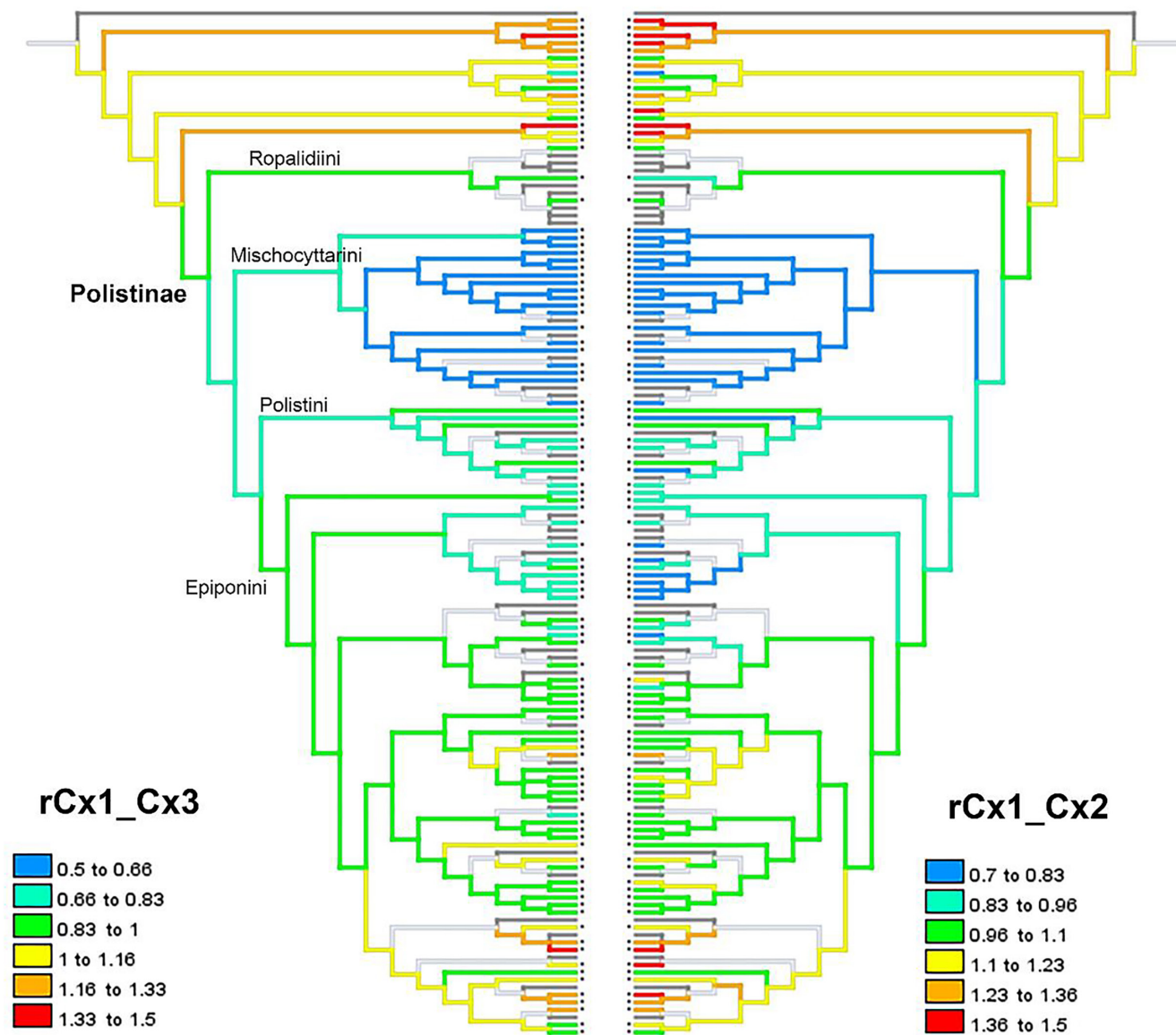


FIGURE 11 Mirrored trees for studied Vespidae with mappings of ancestral values for the coxae ratio variables “rCx1_Cx3” (lengths of forecoxa/hindcoxa; left tree) and “rCx1_Cx2” (lengths of forecoxa/midcoxa; right tree) based on weighted squared change parsimony (WSCP; with Mesquite 3.61). Coloured ranges summarize changes between nodes; species names omitted; unmeasured species in grey (ambiguity in lighter grey).

regarding the execution of several tasks performed in association with the mouthparts (items 14, 15 and 16) in contexts such as nest building, foraging for food and nest materials, social interactions among adult wasps, and the feeding of immatures. Thus, that vespid forelegs (and other aculeate Hymenoptera as well) in addition to the primary locomotory function, also evolved to function as “arms” to be used in the handling of diverse types of materials and in social interactions cannot be considered a novel insight. Close spatial and functional association with mouthparts appear to have been a factor influencing the relatively small size of forelegs in vespids, something also observed in bees and other aculeates. However,

counterexamples of elongated and strong forelegs can be observed in several families, such as Rhopalosomatidae (sister group of Vespidae; Branstetter et al., 2017), Pompilidae and some ants. The relationships between foreleg size and factors like foraging and nesting habits and sociality among aculeates are certainly worthy of a careful investigation.

Such functional independence of the forelegs with respect to the mid and hindlegs is reflected in the patterns of variation of the morphometric variables presented above. The differences are very clear both in respect of size-mediated variation (i.e., different allometric patterns; see Figure 4), as well as in patterns dictated

TABLE 7 List of elementary actions or behaviours of female vespids performed mainly with the legs or with their help (respective sources indicated in parentheses; see table footnote for symbol explanation).

1. Walking (horizontal: facing down/up; or vertical/inclined) on rugged or smooth surfaces (a, b, d)
2. Standing (horizontal: facing down/up; or vertical/inclined) on rugged or smooth surfaces (a, b, d)
3. Turning the body (often only on mid and hind legs) (e.g. for construction, social interaction, etc.) (c, j, k)
4. Taking off (legs springing the insect into the air; loss of tarsal contact with the ground) (a, d)
5. Landing (no gradual braking by running forwards: all the shock taken on outstretched legs, endowed with pads, spines and claws for adhesion) (a, d)
6. Stabilizing flight /drag control (a, f, m)
7. Hanging by tarsal claws from nest comb, leaves, twigs, etc. (b, e)
8. Climbing (or landing on) spider webs (e, m)
9. Raising uniformly the body on all legs (e, i)
10. Raising obliquely anterior parts of the body on mid and hind legs (and waving) (e, i)
11. Extreme raising of anterior parts of the body on mid and hind legs (and tip of metasoma) (h)
12. Grasping (or clinging) with all legs around narrow substrates (e)
13. Holding with all legs (holding prey, fighting nest mates) (e, i)
14. Excavating with fore legs (and mandibles) (c, l)
15. Holding and manipulating materials with fore legs (and mandibles; e.g. while depositing pulp in nest construction, or in trophallaxis, or collecting Dufour's gland secretion) (c, i, j, k, n)
16. Pushing (prey) with fore legs (and mandibles, through nest orifice) (c, j, k)
17. Cleaning the body with parts of fore, mid and hind legs (b, c)
18. Mechanical sensing of substrate (tarsal sensilla, tibial subgenual organ) (a,g)

Note: (a) Chapman (1998); (b) Duncan (1939); (c) Evans and Eberhard (1970); (d) Gullan & Cranston (2010); (e) Jeanne (1970); (f) Mikó et al. (2019); (g) Santos et al. (2007); (h) Silveira et al. (2015); (i) West Eberhard (1969); (j) Wasp Builds Unique Nest for Her Young | Trials Of Life | BBC Earth. <https://www.youtube.com/watch?v=fY6m89ILYrs>. channel: BBC Earth; (k) Potter wasp (*Delta conoideum*) mud nest building - Architectural Excellence. <https://www.youtube.com/watch?v=6nhcbwkupz8>. channel: Misc-o-Media; (l) *Odynerus melanocephalus* nest digging. <https://www.youtube.com/watch?v=CBIWe157OpM>. channel: OwlCreekOccurrence. Accessed in 18/10/2021; (m) Hover wasps plucking something off a spider web. https://www.youtube.com/watch?v=M75eVY_GWKE. channel: HB Tang; (n) *Parischnogaster mellyi* (Stenogastrinae). https://www.youtube.com/watch?v=gjmmfcXd_8M. channel: turillazzi. (titles of items j-n as appearing in YouTube, accessed in August/18/2023; accuracy of species identification in these quoted movies is non-essential, tribal or subfamily level is sufficient, i.e., Eumenini for “wasp” or *Delta*; Odynerini for *Odynerus*; and Stenogastrinae for “hover wasp” or *Parischnogaster*).

by phylogeny and independent of size (see regression residuals in Figures 7–10). The distinctive integration properties of the two groups of legs (anterior versus

middle-posterior) are also apparent in the correlation patterns among the measured traits, as shown in the matrices of Tables 4–6.

However, if on one side the correspondence between functional and morphometric aspects can be easily recognized in such differences between the two groups of legs, the same cannot be said of functional counterparts of the other morphometric differences observed across the full range of studied vespid taxa. Evidently, a substantial majority of the behaviours listed in Table 7 can be performed equally well by both long-legged species (e.g., *Mischocyttarus* spp.; Figures 1a,c and 3a) and short-legged species (e.g., *Brachygastra* spp.; Figures 1f and 3b).

4.3 | A model for size effects

Higginson et al. (2015) proposed that evolutionary (interspecific) allometry can be considered a generalized model of trait covariance across species, which can be used to infer modified developmental pathways producing species-specific patterns of covariance. At a time of many substantial advances in the knowledge of the phylogeny of vespid wasps (see Silveira et al., 2021), we propose that the phylogenetic models presented here can be considered as a reference for studies of body-size effects on leg length in social wasps at different hierarchical levels (static or evolutionary allometry).

In the presented (interspecific) regressions for the whole Polistinae (Figure 4), when phylogenetic information was introduced (PGLS), in virtually all cases, there was attenuation of the rates of shape change along the size gradient, with allometric coefficients being lower and closer to 1.0. Only the slopes of lines for regressions of the lengths of mid and hind tarsi remained relatively higher, above 1.10.

In addition to the better statistical performance of these PGLS analyses, the fact that similarly lower allometric slopes were also obtained in separate OLS analyses within polistine subclades (compare values for Polistinae in Figure 4 with those for subclades in Table 3) corroborate the choice of the PGLS models as general explanations for the covariance between body size and leg length in Polistinae.

The preference for the PGLS (instead of OLS) regression models implies in seeing as sampling artefacts some instances of large differences in leg-length proportions in species of different sizes (e.g., in the above-cited *Protopolybia-Polistes* comparison). However, if this is a correct interpretation in respect of the evolutionary origin of polistine traits under the postulated models, in the practical context of the prediction of leg shape of extant

polistines, one should consider that our sample is taxonomically fairly complete, so the OLS regressions might as well be considered efficient descriptors of the scaling relations.

Interestingly, in regressions of mid and hindlegs, both phylogenetic models (PGLS: BM and LAM) apparently adjust to variation of middle-upper data points (mostly *Mischocyttarus* species), but largely “miss” the smallest species (*Leipomeles* and *Protopolybia*), which otherwise are matched by the OLS lines (see Figure 4; Fm2, Tb2, and Fm3, Tb3). In these cases, we see how by taking phylogeny into account (jointly with Brownian motion evolution), the PGLS models end up treating the shapes of the smallest polistines as “unexpected.” However, seen from an evolutionary perspective, this mismatch can be fruitfully interpreted as indicating that the miniaturization in lineages such as *Leipomeles* and *Protopolybia* probably required novel and different adjustments in the scaling relations of legs with body size.

The difference between the two used phylogenetic models resides in that LAM has the extra parameter lambda, which adjusts the lengths of the branches of the phylogenetic tree (Pagel, 1999, 2002) to maximize the fit to the data. This parameter has been commonly reported as one of several indexes of “phylogenetic signal,” which refers to the usual statistical non-independence of species in comparative data (see Blomberg et al., 2003; Münkemüller et al., 2012; Revell et al., 2008). A lambda value equal to 1.0 indicates that the tree branches as proposed and a constant rate Brownian model of evolution would be adequate as the correlation structure for the residual error in the regression models (see Revell & Harmon, 2022). The fact that lambda values for all the regressions (Figure 4) were significantly lower than 1.0 shows instead that a “pure” Brownian model is not the best one (assuming that the tree is correct).

Models used in our study are generally considered as relatively simple (see Pagel, 1999, 2002; Revell & Harmon, 2022), and this is in line with our pattern-oriented objectives. We agree with Pagel (1994), that “Brownian motion is a process (one of many) that can generate normal distributions and so is often invoked for convenience.” We mainly aimed to have some measure of how much a given pattern was a departure from expectation based on ancestry and divergence time. Some of the patterns we found are indeed strong and clearly go against the assumption of a simple homogeneous evolutionary process across the vespid tree. In some branches, evolution seems to have been faster and led to very distinctive shapes as the elongate legs of *Mischocyttarus*, or the very short ones of some epiponine lineages. It is important to note that similar results regarding inferred ancestors’ values were obtained

with “linear” parsimony (see Figures S9–S14), which is not based on probabilistic models.

4.4 | Utility of ratios

Because ratios showed high correlations with the residuals of regressions (Table 4), we used them for inferring the history of the changes in leg shape through the vespid phylogeny. In the Polistinae, there are two more distinctive general patterns of leg elongation or shortening that seems independent of body size. Most species with a stout body tend to have all the leg segments comparatively shorter (Figures 1f,g and 3b). This is probably the most easily recognizable pattern and refers generally to the species in the clades *Pseudopolybia-Parachartergus* and *Brachygastra-Protopolybia*. Despite many solitary vespids presenting similarly stout bodies and short legs, the condition originated independently in the two epiponine social clades. The other distinctive leg-length condition is the opposite of the first and concerns the great elongation mainly of elements of the middle and hind legs, identifiable in species of *Mischocyttarus*, *Polistes* and in the epiponines *Agelaia* and *Apoica* (Figures 1a–c, 9 and 10), but mainly in *Mischocyttarus*. Another shape aspect that makes this latter genus practically unique in Polistinae is that its species present precisely the opposite condition regarding the forelegs, which are very short (Figures 7 and 8; see below).

4.5 | Why the “T-Rex” leg pattern in *Mischocyttarus*?

The proportionally very short forelegs (often flexed and retracted onto the body in nest individuals), sharply contrasting with the very large hindlegs, create an impressive image of *Mischocyttarus* individuals (Figure 1a,c). The extra elongation of mid and hindlegs (Figures 9 and 10) is probably easier to explain in genera of independent founder social wasps, in which dominance is established by physical mechanisms (Jeanne, 1980), and elevated body postures involving the raising of head and forelegs are used by gynes against workers (Jeanne, 1972; West-Eberhard, 1969). Very long mid and hindlegs are also concordant with “raising” behaviours like that described by Silveira et al. (2015), in which individuals raise the front part of their body in an extreme fashion to make the nest (jointly with the wasps) appear like bifurcating dry twigs (see Figure 1c).

However, in relation to the contrasting *Mischocyttarus* very short forelegs, attempts to find a functional explanation based on the social context of independent founders

immediately confront the difficulty that the sociobiologically similar species of *Polistes* (with same general type of nest and also independent founders) show the opposite trend with respect to the relative length of forelegs, these generally being longer than expected based on species size (Figures 7 and 8).

As alternative to functional explanations, one might (intuitively) speculate that the extra reduction in length of forelegs of *Mischocyttarus* could represent a kind of trade-off response to the exceptional elongation of the mid and hind legs. Developmental studies on holometabolous insects have shown that the final size of adult appendages results from cell proliferation in imaginal discs during part of the last larval stage (prepupa), after larval feeding has already ceased (Emlen & Allen, 2004; Emlen & Nijhout, 2000). In such a “closed system,” a model developed by Nijhout and Wheeler (1996) of “imaginal disk competition” predicted that when one disk is reprogrammed, others will show a compensatory response. Experimental work with beetles and butterflies indeed has demonstrated that changes in the allocation of developmental resources to a given trait (horn, wing) produced such compensatory changes in the relative sizes of a different trait (Nijhout & Emlen, 1998). The possibility of extrapolation of such developmental phenomena to the evolutionary level has been pointed out by both Nijhout and Wheeler (1996) and Nijhout and Emlen (1998), that is, that such correlated developmental responses may play a role in the evolution of body proportions and morphological integration of traits.

A further argument in respect of the legs of *Mischocyttarus* relates to a special condition of the mid and hind tarsi, which present notably asymmetrical lobes and terminal claws, the internal longer than the external ones. This has been traditionally recognized as a diagnostic feature of the genus, unique in Polistinae (Silveira, 2008), and to which Jeanne (1972) even attributed an adaptive role related to the ability of the wasps to climb spider webs. Tarsal asymmetry is evidently correlated with the differential lengthening of mid and hind legs (since forelegs are short and have symmetrical tarsal lobes and terminal claws), and it seems possible that there is some causal basis for this relation, either developmental or adaptative.

4.6 | Solitary vespids

Our sample of species of solitary subfamilies is very small, especially regarding the very speciose and diverse Eumeninae. Most of the examined solitary species have mid and hindlegs shorter than those of most social polistines and vespines, except for those in the eumenine

clade *Zeta-Pachymenes* that have “medium-length” legs (Figures 10 and 11). Stenogastrinae species have leg proportions that are more similar to those of solitary groups, that is, shorter than those of the other two eusocial subfamilies.

Another feature observed in solitary taxa that may be of some significance refers to the relatively long (and often broad) forefemur of euparagiines, masarines (less so in *Gayella eumenoides*), the two studied *Zethus*, and the *Pachodyneus* species (Figure 7). Such a proportional feature also results from comparisons between mid and fore femora, which result in ratios lower than 1.0 in all these species. The pattern for a ratio involving the mid and fore tibiae was similar. In Masarinae, this mid/foreleg proportion pattern is generally complemented by the forecoxa much larger than the midcoxa. It is possible that fossorial habits are involved in the origin of these leg proportional configurations.

4.7 | Effects of using the *bladj* method for tree node height adjustment

While several methods exist to compile or synthesize phylogenetic topological information from different sources to use in comparative studies, producing estimates of branch lengths for the resulting composite trees remains a difficulty (Beaulieu et al., 2012). One solution for missing branch lengths that have been commonly used (especially in plant ecology) is branch-length adjustment, wherein true age estimates are available for some tree nodes and (unknown) ages of remaining nodes are evenly distributed (e.g., the *bladj* algorithm in Phylocom; Webb et al., 2011). Molina-Venegas and Rodríguez (2017) in a simulation-based study examined the extent to which such “pseudo-chronograms” could affect estimates of phylogenetic signal. They concluded that while pseudo-branch lengths (and polytomies) could lead to strong overestimation of phylogenetic signal when using Blomberg et al.’s *K*, Pagel’s *lambda* “seems strongly robust to either incompletely resolved phylogenies and suboptimal branch-length information.” Because we used as backbone tree the molecular phylogeny published by Menezes et al. (2020) which is dated, a fairly large proportion (30%) of the nodes in our composite tree have “true” age estimates so that the analyses may be less susceptible to distortions.

The phylogenetic regression analyses made with the reduced dataset and tree (a “true” chronogram) (Figure S6) resulted very similar to the full data analyses (compare coefficients in Figure 4 and Table 8) not only with respect to the parameter values (between datasets and across traits), but also with the patterns of change

TABLE 8 Parameter values of ordinary (OLS) and phylogenetic (PGLS) linear regressions on a reduced leg-measurement dataset and pruned tree (Figure S6) of the Polistinae, for six length variables of fore, mid and hind femora and tibiae (respectively Fm1, Tb1, Fm2, Tb2, Fm3 and Tb3) on a size variable (height of mesopleuron, Mpl).

Leg element	OLS				PGLS				
	<i>a</i>	<i>b</i>	<i>SE</i>		<i>a</i>	<i>b</i>	<i>SE</i>		<i>Lambda</i>
FM1	−0.2739	1.1296	0.0319	**	−0.1773	1.0697	0.0335	*	0.8748
TB1	−0.3705	1.1347	0.0299	**	−0.3044	1.0957	0.0335	**	0.6660
FM2	−0.3352	1.2030	0.0378	**	−0.1202	1.0845	0.0375	*	0.8494
TB2	−0.5150	1.3021	0.0508	**	−0.2039	1.1287	0.0445	**	0.9672
FM3	−0.3044	1.2230	0.0377	**	−0.0895	1.1046	0.0354	**	0.8958
TB3	−0.5129	1.3702	0.0558	**	−0.1429	1.1664	0.0498	**	0.9586

Note: *a*: intercept; *b*: slope; *SE*: slope standard error; lambda: Pagel's branch-length scaling parameter.

between the OLS and PGLS analyses being virtually identical. The values of lambda of the PGLS are also quite alike between datasets, noting the precise coincidence of the lowest values being obtained for the regression of the foretibia length (Tb1).

We see this as evidence that using the “bladj” adjustment for heights (ages) of many internal nodes of the larger Vespidae tree has not introduced significant noise in the analyses. Without the use of bladj, the study would lose the information of 72 [!] polistine species, most from the genera *Mischocyttarus*, *Polistes*, and the tribe Ropalidiini. Some effects of data loss can be appreciated in the Figure S6 (with the parsimonious optimization of the ratio rTb3; hindtibia length/mesopleuron height), where leg-shortening patterns in Epiponini are still reasonably well demonstrated, but the very peculiar patterns of *Mischocyttarus* (with only three species) lose strength and sharpness when compared to other Polistinae.

4.8 | Effects of alternative phylogenetic position of the Stenogastrinae

We treated the Stenogastrinae as sister group to the other two eusocial vespidae subfamilies after Carpenter (1982) and Pickett and Carpenter (2010). However, adopting the topological relations indicated by Piekarski et al. (2018) for Stenogastrinae and some other vespidae subfamilies (i.e., with basal Stenogastrinae, and paraphyletic Masarine and Eumeninae) had not produced significant differences on ancestral values for the leg morphometric characters in the major eusocial clade Vespinae + Polistinae. Because the phylogenomic paper of Piekarski et al. (2018) did not provide divergence times for the lineages, there is no specific age estimate available for a basal split between the Stenogastrinae and postulated sister lineage (encompassing the remaining vespidae taxa). We tried linear parsimony optimization (with TNT v. 1.5) of the

morphometric characters on an “equal branch-lengths” version of Piekarski's phylogenetic hypothesis for the Vespidae (see Figures S15–S20). The drastic topological changes on the base of this tree accordingly result in different histories for the leg traits in this basal sector of the tree, but the patterns of changes on the part of the tree involving the eusocial Vespinae and Polistinae are very similar to those presented before.

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