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Research article

Shape differences rather than size differences between castes in the Neotropical swarm-founding wasp *Metapolybia docilis* (Hymenoptera: Vespidae, Epiponini)

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Abstract

Background: Swarm-founding epiponine wasps are an intriguing group of social insects in which colonies are polygynic (several queens share reproduction) and differentiation between castes is often not obvious. However, caste differences in some may be more pronounced in later phases of the colony cycle.

Results: Using morphometric analyses and multivariate statistics, it was found that caste differences in *Metapolybia docilis* are slight but more distinct in latter stages of the colony cycle.

Conclusions: Because differences in body parts are so slight, it is proposed that such variation may be due to differential growth rates of body parts rather than to queens being larger in size, similar to other previously observed epiponines.

Background

Polistine wasps in the tribe Epiponini are remarkable for their polygyny, swarm-founding habits, and reputed slight differentiation between castes. Although the overall features of epiponines are well-documented and discussed [1–3], many sociobiological details remain obscure. For example, for decades, swarm-founding polistines have been considered permanently polygynous, even though monogyny has been detected in some species [1,4,5]. An explanation was offered by [6], who showed that *Metapolybia aztecoides* alternates between polygyny and oligogyny and, eventually monogyny in the course of its life cycle. This cyclical oligogyny [6] seems to be important in the maintenance of high genetic relatedness in epiponine wasps [7–9]. In fact, new queens are produced with just one queen in the nest [10] in some species.

Preliminary overall studies [1,4] demonstrated a wide range of queen-worker dimorphism among different taxa in the Epiponini. Three different forms of queen-worker distinction were recognized [1], but, recently, five forms of caste differentiation due to the influence of the colony cycle were recognized [11]. They are: 1, the absence of morphological differences between queens and workers during the entire colony cycle (*Parachartergus smithii* [12], *Pseudopolybia vespiceps* [13], *Chartergellus communis* [14], *Brachygastra lecheguana* [15]); 2, the absence of morphological differences between castes, with some young females developing ovaries in only some phases of the colony cycle (*Synoeca cyanea* [16]); 3, morphological discontinuities between castes related to variation during the colony cycle, with non-inseminated laying females present during the entire colony cycle (*Protopolybia exigua*

and *P. sedula* [11]); 4, caste differences increase during the colony cycle with non-inseminated laying females occurring only some phases of the colony cycle(*Chartergus globiventris* [11], *Polybia scutellaris, P. occidentalis*, and *P. paulista* [17], *Protonectarina sylveirae* [11], *Epipona guerini* [18]) and; 5, queens always distinct from workers, with no variation in this pattern occuring during the colony cycle (*Agelaia areata* [19], *A. vicina* [20,21], *A. pallipes* and *A. multipicta* [22], *Apoica flavissima* [23] and *A. pallens* [24], *Polybia dimidiata* [25]).

Even though *Metapolybia* may be considered a model genus for studies related to colony population structure in epiponines, just a few studies (taxonomy – [26], ovary development – [27], colony composition, including caste differences [28] and nest construction – [29]) have been performed on this taxon since [6], despite the increase in knowledge of this tribe. This paper examines caste differences and other ecological aspects of *Metapolybia docilis*.

Results
Nests and related aspects
Colonies under investigation were assigned to one of 4 stages, after [17] (Table 1): (a) pre-emergence of workers, in which no adult offspring had been produced (one colony); (b) emergence of workers, with different-aged brood and at least one adult generation (one colony); and (c) male producing (one colony). Also, two other colonies were migrating for a new location. This fourth stage was defined as (d) swarm or absconding (two colonies). Even though these two forms of swarm are difficult to distin-

guish, behavioral observations (unpublished data) indicated that only part of the population migrated to a new nest in the *swarm* colony. In addition, swarming queens were exclusively younger females (see below) suggesting production of sexuals in the mother nest. The absconding colony represents the migration of a whole colony after some disturbance once the nest was damaged. In addition all queens were old (see below) evidencing that this colony was not producing new queens, representing the end of a cycle.

Females' ovary condition, insemination and their relative age
Two types of ovarian development were recognized as type A, with filamentous ovarioles bearing no visible or slightly developed oocytes, and type B, with well developed and longer ovarioles, each one bearing from two to several mature oocytes. Since only type B females contained sperm in the spermatheca, females with type A and B ovarian development can be characterized as workers and queens, respectively.

According to our data, the colony cycle in *Metapolybia docilis* is characterized by great age variation (Figure 1). In the pre-emergence colony both workers and queens varied substantially in age. In worker-producing and male-producing colonies most workers were younger and most queens were older. In the absconding colony, workers varied in age, but the single queen was old (black pattern). In the swarm colony, queens and workers were young and old individuals, respectively, without intermediate age.

Table 1: Number of combs, brood production, and colony population in the analyzed colonies of *Metapolybia docilis*. Percentage in parentheses.

| Nest Location	Colony phase	Cells					Estimated n. of generations	Females		Males
		Empty	with eggs	With larvae	With pupae	total		Workers	Queens	
Igaçaba – São Paulo State	Worker-producing	123	108	268	321	820	1	197	23	0
		(15.0)	(13.2)	(32.7)	(39.2)			(89.6)	(10.5)	
Igaçaba – São Paulo State	Male-producing	313	42	26	6	387	2	165	4	7
		(79.9)	(10.9)	(6.7)	(1.6)			(93.8)	(2.3)	(3.98)
Pedregulho – São Paulo State	Pre-emergence	41	19	0	0	60	0	64	9	0
		(68.3)	(31.7)					(87.7)	(12.3)	
Nova Xavantina – Mato Grosso State	absconding	-	-	-	-	-	-	44	1	0
								(97.8)	(2.2)	
Ribeirão Preto – São Paulo State	Swarm	-	-	-	-	-	-	11	7	0
								(61.1)	(38.9)	
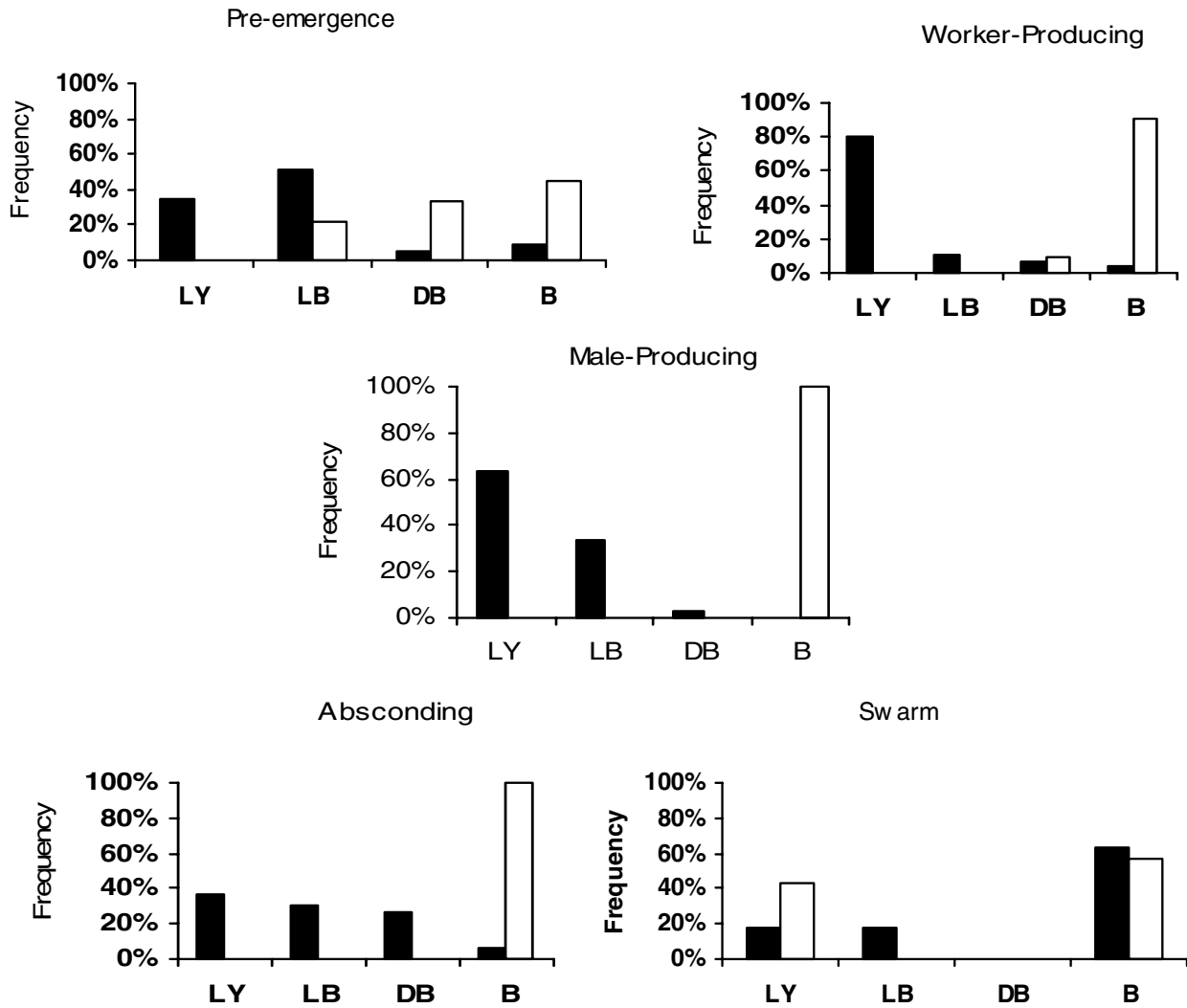
Figure 1
Frequency of queens (White bars) and workers (dark bars) according to the color patterns of the transverse apodeme from the 4th gastral sternite in *Metapolybia docilis* in different analyzed colonies. LY – light yellow, LB – light brown, DB – dark brown, B – black.

Morphological differences

Mean differences of 10 measured characters were tested in workers and queens by Bonferroni *t*-test (Table 2). In all analyzed colonies queens were hardly distinct from workers. Univariate statistics showed that just a few characters for each colony were different among queens and workers (Table 2) in all colonies. Except for MTL in a worker-producing colony, all significant differences showed queens slightly larger than workers. Using the characters with significant differences in univariate statistics, covariance analysis (ANCOVA: Table 2) showed statistically signifi-

cant values in all these characters except for T1L in the pre-emergence colony, demonstrating that these characters differed in size and body proportions. ANCOVA was not tested in the absconding colony due to the presence of only one queen.

Discriminant function analysis (Table 3) showed also that only a few characters used in the model resulted in significant *p*-values. Wilk's Lambda values ranged from 0.64 to 0.8 (Table 10) in pre-emergence and worker-producing colonies and from 0.13 to 0.75 (one variable only) in

Table 2: Queen and worker means (in mm), Bonferroni *t*-test and ANCOVA analyses for each morphometrical variable in *Metapolybia docilis*.

Colony Phase	Characters	Means ± SD		Bonferroni t-test		ANCOVA (AL as covariate)			
		Queens	Workers	F	P>F	MS Effect	MS Error	F	*p*-level
Pre-emergence	9 queens and 64 workers								
	HL	2.00 ± 0.06	1.95 ± 0.07	1.89	0.06				
	HW	2.42 ± 0.06	2.41 ± 0.07	0.63	0.53				
	IDm	1.00 ± 0.04	0.99 ± 0.04	0.25	0.80				
	PW	1.86 ± 0.07	1.83 ± 0.06	1.79	0.08				
	MTL	0.48 ± 0.03	0.47 ± 0.03	0.72	0.48				
	AL	2.97 ± 0.12	2.90 ± 0.14	1.53	0.13				
	T2H*	2.02 ± 0.06	1.90 ± 0.10	3.19	0.002	0.05	0.01	7.91	0.01
	T1L*	2.22 ± 0.04	2.16 ± 0.08	2.20	0.03	0.01	0.003	2.41	0.13
	T2BW	0.50 ± 0.02	0.49 ± 0.04	0.77	0.45				
	WL	4.48 ± 0.10	4.40 ± 0.13	1.72	0.09				
Worker-producing	23 queens and 30 workers	HL	2.03 ± 0.04	2.02 ± 0.03	1.66	0.10			
	HW	2.44 ± 0.04	2.46 ± 0.05	-1.26	0.21				
	IDm	1.04 ± 0.02	1.04 ± 0.03	1.58	0.12				
	PW	1.28 ± 0.04	1.29 ± 0.07	-0.53	0.60				
	MTL*	0.50 ± 0.02	0.53 ± 0.03	-3.00	0.004	0.01	0.001	11.40	
	AL	3.25 ± 0.07	3.24 ± 0.09	0.60	0.55				
	T2H*	2.11 ± 0.08	1.96 ± 0.07	7.55	<0.001	0.31	0.005	62.74	
	T1L	2.25 ± 0.04	2.23 ± 0.06	1.44	0.16				
	T2BW	0.51 ± 0.02	0.50 ± 0.02	1.74	0.09				
	WL	4.56 ± 0.08	4.54 ± 0.10	0.62	0.54				
Male-producing	4 queens and 30 workers	HL	1.95 ± 0.05	2.00 ± 0.04	1.98	0.06			
	HW	2.43 ± 0.07	2.45 ± 0.03	0.82	0.42				
	IDm	1.03 ± 0.03	1.03 ± 0.03	0.12	0.90				
	PW*	1.42 ± 0.05	1.27 ± 0.04	-5.69	<0.001	0.08	0.002	41.39	<0.001
	MTL	0.54 ± 0.03	0.53 ± 0.03	-0.80	0.43				
	AL	3.06 ± 0.15	3.15 ± 0.09	1.73	0.09				
	T2H	1.98 ± 0.07	2.02 ± 0.11	0.60	0.55				
	T1L	2.20 ± 0.12	2.23 ± 0.05	1.02	0.32				
	T2BW	0.50 ± 0.00	0.48 ± 0.02	-0.85	0.40				
	WL	4.53 ± 0.16	4.55 ± 0.09	0.41	0.69				
Absconding	1 queen and 44 workers	HL	2.00	1.94 ± 0.05	1.10	0.28			
	HW	2.37	2.40 ± 0.05	-0.67	0.51				
	IDm	1.06	1.02 ± 0.05	0.87	0.39				
	PW	1.25	1.24 ± 0.08	0.05	0.96				
	MTL*	0.56	0.47 ± 0.04	2.19	0.04				
	AL	3.06	3.06 ± 0.11	0.02	0.99				
	T2H	2.00	1.95 ± 0.10	0.49	0.63				
	T1L	2.18	2.15 ± 0.07	0.39	0.70				
	T2BW	0.50	0.49 ± 0.03	0.13	0.90				
	WL	4.25	4.31 ± 0.11	-0.57	0.57				
Swarm	7 queens and 11 workers	HL	1.94 ± 0.04	1.92 ± 0.06	1.03	0.32			
	HW	2.37 ± 0.05	2.34 ± 0.08	0.87	0.40				

Table 2: Queen and worker means (in mm), Bonferroni *t*-test and ANCOVA analyses for each morphometrical variable in *Metapolybia docilis*. (Continued)

IDm*	1.01 ± 0.03	0.97 ± 0.03	2.69	0.02	0.01	0.001	9.03	0.01
PW	1.19 ± 0.04	1.18 ± 0.07	0.29	0.78				
MTL	0.50 ± 0.00	0.48 ± 0.03	1.18	0.26				
AL	3.00 ± 0.03	2.96 ± 0.03	0.50	0.63				
T2H*	1.94 ± 0.07	1.82 ± 0.08	3.28	0.005	0.06	0.01	9.71	0.01
TIL	2.15 ± 0.03	2.14 ± 0.09	0.11	0.91				
T2BW	0.48 ± 0.03	0.47 ± 0.03	0.69	0.50				
WL*	4.41 ± 0.07	4.26 ± 0.14	2.62	0.02	0.07	0.01	10.61	0.01

* Statistically significant

male-producing and swarm colonies. This indicates that in the former some individual characters were not good enough to discriminate castes, and caste differences could be determined only in association with these characters, which demonstrates low differentiation between castes (Fig. 2). In the latter, however, some characters appear to have a better power of discrimination (Fig. 2). Such differences suggest that variation during the colony cycle may occur as in other epiponines [11,17]. In fact, comparing actual groups with predicted groups through discriminant analysis (Table 3), it is evident that queens form more distinct groups in the later stages of the colony cycle. These results emphasize how queens, apart from being hardly distinguishable by size, exhibit some differences that are perhaps more related to shape than size.

Discussion

In *Metapolybia*, castes are hardly detectable by external morphology [1,28], and they are apparently determined by disputes among adults rather than by larval manipulation [6,30]. For this reason, young females have their ovaries suppressed in queen-right colonies, and only in orphanage young females develop their ovaries and become queens [6]. In our study, *Metapolybia docilis* exhibits slight differentiation between queens and workers, at least in the early stages of nest development, as compared to other taxa such as *Agelaia* spp. (*A. flavipennis* [31]; *A. areata*, [19]; *A. pallipes* and *A. multipicta* [22]; *A. vicina* [20,21]) that have a clear-cut caste differentiation. Also, the clear physiological distinction found between queen and worker ovaries (Fig. 2) suggest strong control of reproduction.

Group comparisons (Table 3) showed queens and workers as unique, well-defined groups, in the latter stages of the colony cycle. In addition, Wilks' Lambda values were relatively low for such colonies (Table 3), demonstrating that, in these colonies, castes can be relatively well-recognized by morphology in the later stages of the colony cycle. Such a pattern seems to be widespread for the Epiponini [11,17], and it is suggested that the increase in castes differences is probably due to the elimination of the

smaller queens. The fact that queens in the later stages of the colony cycle are slightly more distinct from those found in early stages suggest that the phenomenon of queen-selection [6] during the colony cycle may have a morphological component similar to other species previously studied [11,17]. In *Synoeca cyanea* [16], a similar pattern was identified. However, intermediate females were found in some colonies and differences between castes were always absent.

Differently from other species with increasing caste differences according to colony cycle, in *M. docilis*, size differences are very slight. Based on the plots of figure 2, castes are always intersecting each other, and in figure 2A, the growth rates, represented by different slopes detected by ANCOVA analyses (Table 2), are different between castes. Such results indicate that queens and workers differ more in shape than in size. Shape differences have been previously detected for *Apoica pallens* [24] but, in this case, it was accompanied by size differences, as in other species, such as *Epipona guerini* [18] and *Pseudopolybia difficilis* [32]. According to [24] it is possible that the first step of caste evolution in epiponines may be related to changes in body proportions rather than in size due to an ontogenetic reprogramming in the growth parameters (see also [33]). Thus, the almost absence of queen-worker size differences in *Metapolybia* illustrates how complex castes in epiponines is.

Low morphological differentiation accompanied by high physiological distinction was suggested for *Synoeca* and *Metapolybia* [6,30]. In these cases, only certain females that are already mated become queens. During cyclical oligogyny, a low number of queens would signal that newer females would "take a chance" to become new queens. After queen repopulation, orphanage behaviors cease, and workers use aggressive behaviors to inhibit other new females from becoming queens. In this case, non-inseminated ovarian-developed females, common in the some epiponines [11] are completely suppressed during the entire colony cycle due to worker policing as suggested by [11]. In fact, queens' age (Fig. 1) reinforces such
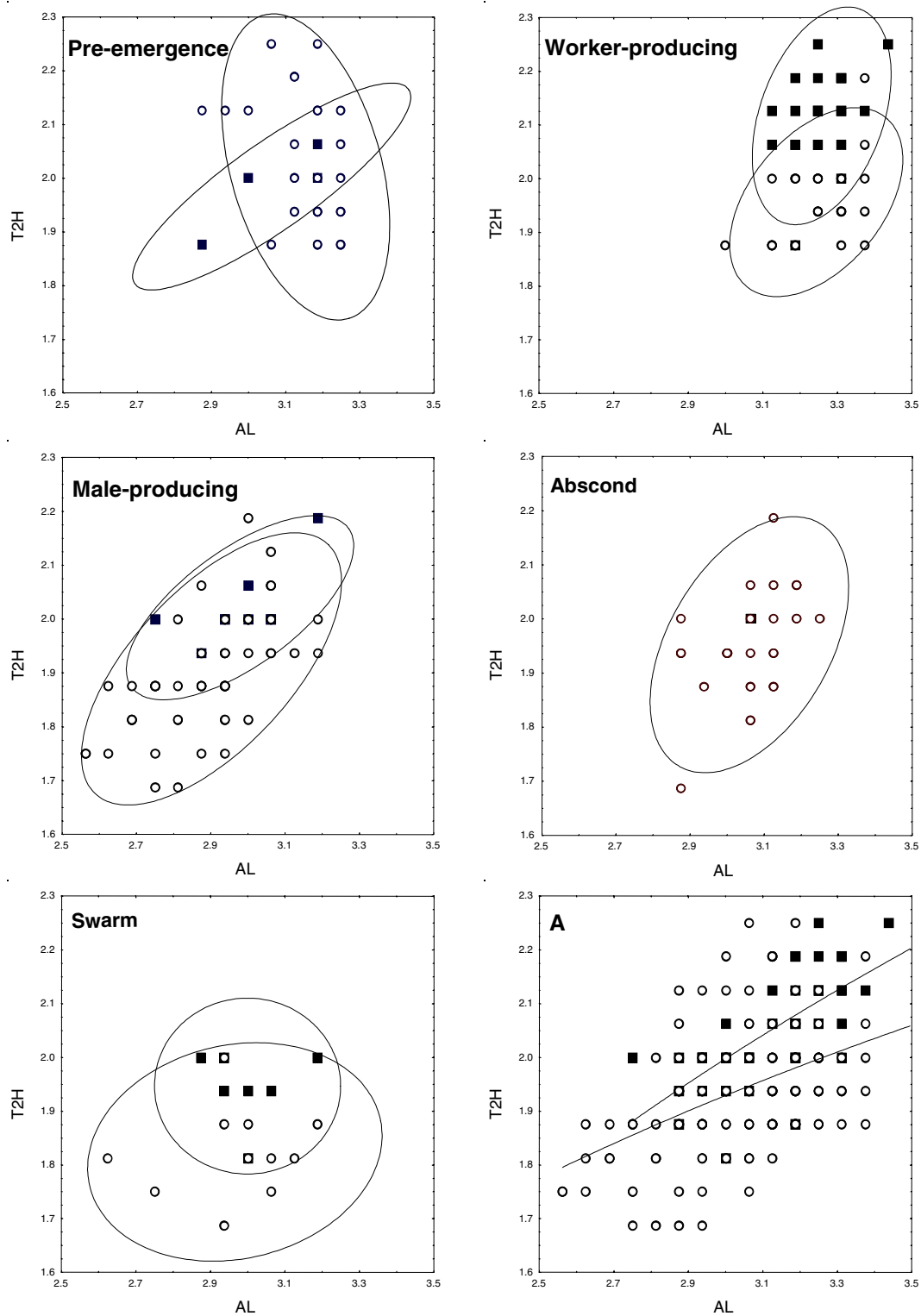
Figure 2
Plots (in mm) of alitrunk length (AL) versus basal height tergum II (T_2H) in five colonies of *Metapolybia docilis*. Queens = black squares; Workers = white circles. Each ellipse encompasses 95% of the variation found in each group. A – discrimination between all queens and workers of *Metapolybia docilis* using the log-log plots.

Table 3: Discriminant morphometric variables between queens and workers in *Metapolybia docilis* based on discriminant function analyses using the stepwise procedure and classification results for group comparisons through discriminant analysis. Original data are based on mm.

Colony Phase	Variable	Step	Wilks Lambda	F	p	Observed classification	Predicted classification		Percent correct
Pre-emergence	T2H*	1	0.80	10.17	<0.001	Worker	61	3	95.31
	HW*	2	0.75	5.70	0.02	Queen	3	6	66.67
	PL	3	0.70	3.50	0.07	Total	64	9	91.7
	T2BW	4	0.69	3.53	0.06				
	TIL	5	0.66	2.88	0.09				
	MTL	6	0.67	2.70	0.10				
	IDm		0.66	2.98	0.09				
	HL		0.64	1.20	0.28				
	HL		0.64	1.20	0.28				
	AL		0.64	1.16	0.29				
Worker-production	T2H*	1	0.80	57.00	<0.001		Worker	Queen	
	HW*	2	0.75	6.25	0.02	Worker	29	1	96.7
	HL	3	0.70	3.61	0.06	Queen	3	20	86.96
	MTL	4	0.69	3.03	0.09	Total	32	21	92.45
	IDX	5	0.66	1.71	0.20				
	TIL	6	0.67	2.40	0.13				
Male-production									
	PW*	1	0.75	32.34	<0.001	Worker	30	0	100
	TIL*	2	0.29	9.00	0.01	Queen	0	4	100
	HL	3	0.30	2.76	0.11	Total	30	4	100
	T2BW	4	0.32	2.74	0.11				
	AL		0.29	1.52	0.23				
	IDM		0.30	1.95	0.17				
	HW		0.29	1.24	0.28				
	T2H*	1	0.25	10.75	<0.001	Worker	11	0	100
Swarm	WL	2	0.37	3.66	0.08	Queen	0	7	100
	TIL*	3	0.18	8.43	0.01	Total	11	7	100
	PL	4	0.23	2.17	0.16				
	HL	5	0.24	3.92	0.07				
	HW*	6	0.19	5.40	0.04				
	IDm	7	0.13	1.01	0.34				

assumption, once pre-emergence colonies initiate with differently aged queens, and only older queens are found in worker-producing and male-producing colonies. In swarming colonies, the queen population is composed either of very young or very old individuals, suggesting that new reproductive are produced only during discrete windows of time.

The fact we found some distinction between castes would not reject the possibility of some young individuals (potential workers) may try to become queens [6,30]. Their options would be two-fold: they could lead the colony until a new queen repopulation occurs, or they could compete with those true queens during the early stages of the colony cycle. However, they would probably be removed from the colony as the cycle progresses [11].

Conclusions
As stated by [4] and [31], because epiponines frequently migrate, development of true castes would not be advantageous. This assertion may be correct in part, once castes are hard to detect. However, differently from the other "highly eusocial insects" (bees, ants, termites and vespine wasps) which bear pronounced size dimorphism, caste differences in epiponines evolved in such a way that imperceptible size differences are expected in most species, probably due to reprogramming of growth parameters [24]. The pattern of caste differences found in *Metapolybia docilis* supports this hypothesis.

Methods
Five colonies of *Metapolybia docilis* used in this work were collected at nightfall. They were collected into a plastic

bag containing ether-moistened cotton balls. Populations were fixed in Dietrich's solution and then kept in 70 % ethanol for dissection. In order to detect morphological differences between the castes, the following 10 external body parts were measured for each specimen under a binocular microscope; head width (HW), head length (HL), minimum interorbital distance (IDm), pronotum width (PW), mesoscutellum length (MTL), alitrunk length (AL), length of gastral tergite I (T1L), basal width of gastral tergite II (T2BW), height of T2 (T2H) and partial length of the forewing (WL) (see also Fig. 2 in [23] more details about these measurements). We examined ovarian conditions by dissecting all individuals under a stereomicroscope. In order to analyze insemination, the spermatheca was removed and put on a slide in a 1:1 solution of glycerin and alcohol (70%). The presence of sperm cells was detected by using a compound microscope. Numerical data were statistically analyzed in relation to the ovarian and spermathecal states.

Before statistical analysis, data were log transformed. Means and standard deviations were calculated from the ten morphological measurements. The Bonferroni *t* test was used for mean comparisons. The contribution of each variable to caste discrimination was examined using stepwise discriminant function analysis [34]. In this method, variables are successively added to the model based on the higher *F to enter* values; variable addition ceases when the F-ratio is no longer significant. Wilks' Lambda values were used to infer the individual contribution of each variable to the model. ANCOVA was used with alitrunk length (AL) as a covariate based on its efficiency in previous publications [18,24,32].

In order to assess the age of females, progressive pigmentation of the transverse apodeme was analyzed. The patterns used here were those that, according to [4,35] indicate a progressive increase in age. They were: LY (light yellow) for the youngest individuals, LB (light brown), DB (dark brown) and B (black) for the oldest individuals.

Authors' contributions

MVB participated in morphometric analyses, females' dissections. FBN and RZ participated in the design of the study, performed the statistical analysis and wrote the manuscript. All authors read and approved the final manuscript.

Acknowledgments

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