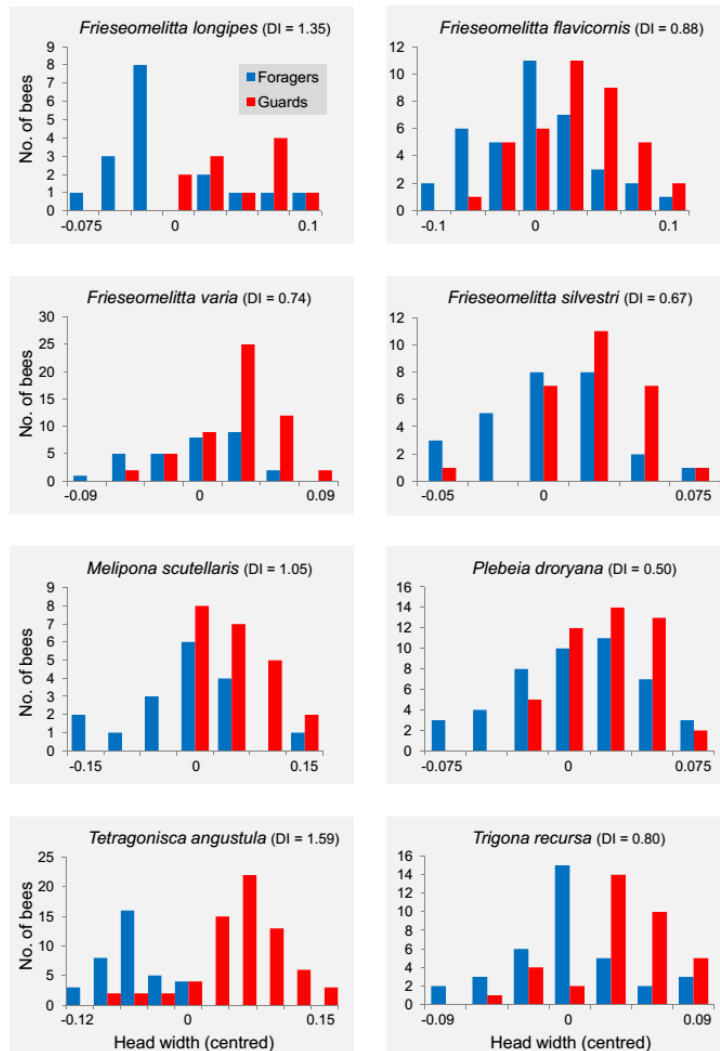
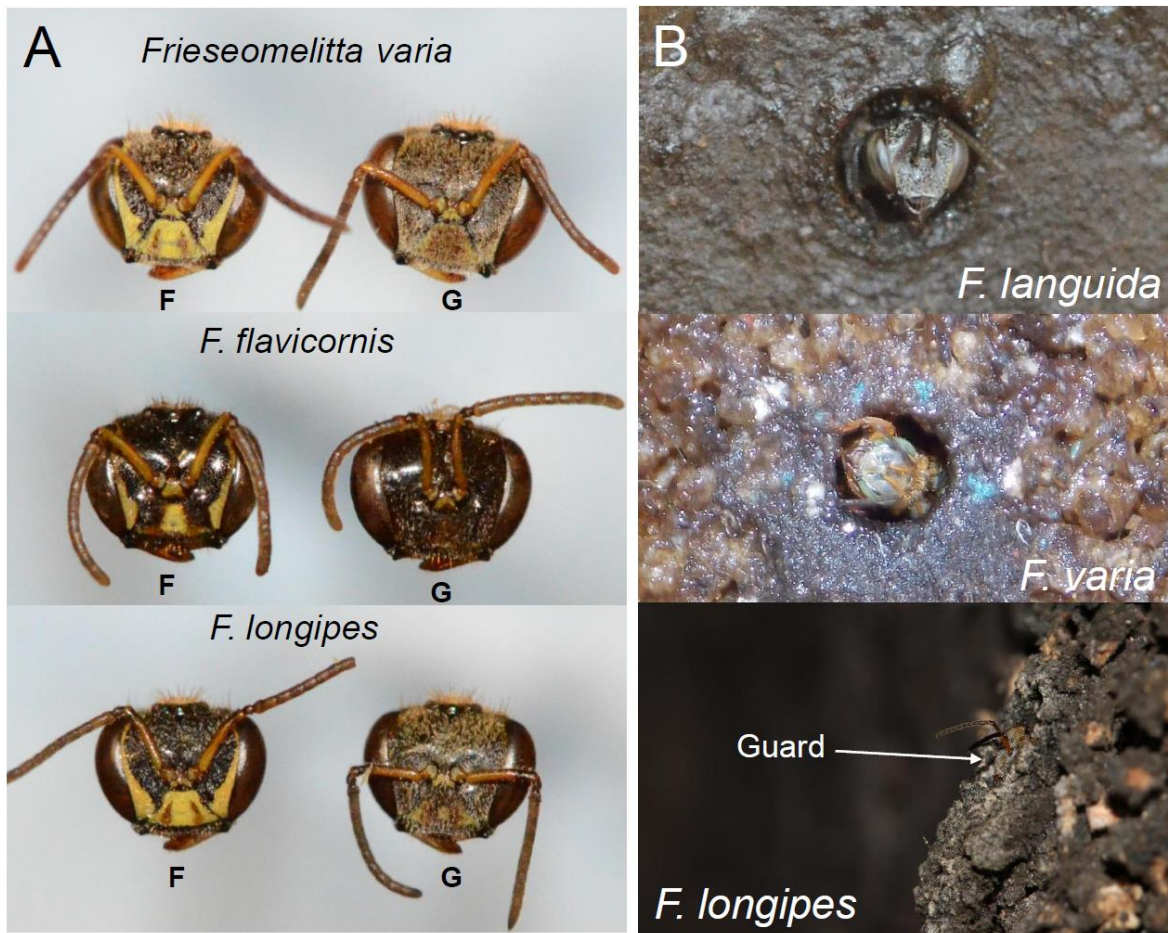


**Supplementary Table 1. Samples sizes, location of collection and information about the natural history of the 28 studied species.** No traffic data for both measuring periods was available for *Scaura latitarsis* due to the remoteness of the sampling location. Queen-worker dimorphism (Q-W ratio) is provided for species where information is available.

Species	Nesting type	Brood comb type	Colonies	Bees	Traffic/1min	Q-W ratio	Origin of colonies
<i>Friesella schrottkyi</i>	cavity	combs	8	82	3.3	1.5	Ribeirão Preto - São Paulo
<i>Frieseomelitta flavicornis</i>	cavity	cell cluster	6	76	1.2	2.03	Belém - Pará
<i>Frieseomelitta longipes</i>	cavity	cell cluster	4	28	1.8		Belém - Pará
<i>Frieseomelitta silvestrii</i>	cavity	cell cluster	5	54	9.0		Nova Xavantina - Mato Grosso
<i>Frieseomelitta varia</i>	cavity	cell cluster	6	86	10.6	1.3	Ribeirão Preto - São Paulo
<i>Geotrigona mombuca</i>	ground	combs	2	44	3.8		Ribeirão Preto - São Paulo
<i>Lestrimelitta limao</i>	cavity	combs	2	72	25.0		Ribeirão Preto - São Paulo
<i>Leurotrigona muelleri</i>	cavity	cell cluster	3	60	5.9		Ribeirão Preto - São Paulo
<i>Melipona fasciculata</i>	cavity	combs	6	75	1.8	1.26	Belém - Pará
<i>Melipona flavolineata</i>	cavity	combs	5	61	2.6	1.34	Belém - Pará
<i>Melipona melanoventer</i>	cavity	combs	6	73	1.8		Belém - Pará
<i>Melipona scutellaris</i>	cavity	combs	5	39	1.7	1.3	Ribeirão Preto - São Paulo
<i>Melipona subnitida</i>	cavity	combs	2	48	0.8	1.2	Mossoró - Rio Grando do Norte
<i>Nannotrigona testaceicornis</i>	cavity	combs	4	102	10.1	1.74	Ribeirão Preto - São Paulo
<i>Paratrigona lineata</i>	ground	combs	2	46	7.7		Ribeirão Preto - São Paulo
<i>Partamona helleri</i>	cavity	combs	2	61	22.1		Ribeirão Preto - São Paulo
<i>Plebeia droryana</i>	cavity	combs	5	92	8.5	1.9	Ribeirão Preto - São Paulo
<i>Scaptotrigona bipunctata</i>	cavity	combs	2	94	32.8	2.09	Ribeirão Preto - São Paulo
<i>Scaptotrigona aff. depilis</i>	cavity	combs	4	76	31.6	1.5	Ribeirão Preto - São Paulo
<i>Scaptotrigona tubiba</i>	cavity	combs	2	69	8.0		Belém - Pará
<i>Scaura latitarsis</i>	termite nest	cell cluster	3	55			Belém - Pará
<i>Tetragona clavipes</i>	cavity	combs	3	69	91.2		Ribeirão Preto - São Paulo
<i>Tetragonisca angustula</i>	cavity	combs	6	105	27.4	3.3	Ribeirão Preto - São Paulo
<i>Tetragonisca fiebrigi</i>	cavity	combs	6	123	33.6		Santa Helena - Paraná
<i>Trigona fuscipennis</i>	cavity	combs	2	90	269.5		Ribeirão Preto - São Paulo
<i>Trigona hypogea</i>	cavity	combs	2	76	80.0		Ribeirão Preto - São Paulo
<i>Trigona recursa</i>	cavity	combs	3	72	48.6		Ribeirão Preto - São Paulo
<i>Trigonisca nataliae</i>	cavity	cell cluster	2	44	4.5		Nova Xavantina - Mato Grosso



**Supplementary Figure 1. Size-frequency distribution (head width) of foragers and guards of eight species with significantly larger guards.** Head width data were centred (for each colony separately). Size distribution is bimodal in *Tetragonisca angustula* and *Frieseomelitta longipes*, indicating the existence of two distinct physical sub-castes. The DI (differentiation index) measures the degree of differentiation between guards and foragers in relation to overall worker size variation. For sample size information, please consult Supplementary Table 1.



**Supplementary Figure 2.** (A) Head of guards and foragers in three different *Frieseomelitta* species. The differentiation indexes (DI) for melanisation were 1.7 in *Frieseomelitta varia* (Fig. 2c), 1.5 in *F. flavicornis* and 1.85 in *F. longipes*. These were the highest DI's we found (including DI's found for size differences), indicating the existence of two distinct colour morphs (Photos: C. Grüter). (B) *Frieseomelitta languida*, *F. varia* and *F. longipes* entrance holes. Entrance holes in *Frieseomelitta* are amongst the smallest in stingless bees. The darker species *F. languida* (and *F. silvestrii*) have darker entrances than species with lighter guards (*F. varia* or *F. longipes*). The workers cover the surface surrounding their nest entrance with sticky substances (Photos: C. Grüter and C. Menezes).