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## INTERNATIONALE ENTOMOLOGEN-TAGUNG BASEL 1999

**Comparison between two species in the *Pachycondyla villosa* complex (Hymenoptera: Formicidae)**

by K. Kolmer &amp; J. Heinze

**Zusammenfassung.** Vergleich zweier Arten im *Pachycondyla villosa* Komplex (Hymenoptera: Formicidae). - Individuen der neotropischen Ponerine *Pachycondyla villosa* aus Itabuna (Bahia, Brasilien) wiesen eine große morphologische Heterogenität auf. Aufgrund von unterschiedlichen fixierten Elektromorphen bei fünf Enzymsystemen und morphologischen Unterschieden konnten zwei verschiedene Typen festgestellt werden. Unsere Ergebnisse zeigen, dass es sich hierbei nicht um einen intraspezifischen Polymorphismus, sondern um zwei verschiedene Arten handelt. Der taxonomische Status beider Formen ist noch nicht geklärt.

**Key words.** isozyme electrophoresis - ant taxonomy - *Pachycondyla* - Ponerinae

**Introduction**

The ponerine ant *Pachycondyla villosa* FABRICIUS, 1804 is a widespread and very common species in the neotropics. Its distribution ranges from Texas to central Brazil and Paraguay. The existence of various taxa which are currently all synonymized with *Pachycondyla villosa* indicates that this "species" is very heterogeneous. The enormous morphological variability of *P. villosa* was already pointed out by ROGER (1861), who regarded the species names *bicolor* GUÉRIN-MENÉVILLE, 1844, *pedunculata* F. SMITH, 1858 and *pilosa* F. SMITH, 1858 as junior synonyms of *Pachycondyla villosa*. EMERY (1904, 1911) combined *Ponera inversa* F. SMITH, 1858 and *P. villosa* var. *curvinodis* FOREL, 1899 to *Pachycondyla villosa inversa*. Today, two valid subspecies are recognized: the nominal *P. villosa villosa* and *P. villosa inversa* (BOLTON 1995).

The aim of our study was to clarify the status of morphologically different forms of *P. villosa* from Bahia, Brazil. Morphological heterogeneity in *Pachycondyla villosa* was previously regarded as an intraspecific polymorphism.

**Materials and methods**

**Measurements & morphology.** Founding queens and mature colonies of *Pachycondyla* were collected on the territory of CEPLAC near Itabuna, Bahia, north-eastern Brazil, in March 1998. We examined the external morphology of 60 queens and 60 workers, and in addition dissected the ovaries of 13 queens. Thorax length (pronotum to meta-epinotal suture in dorsal view), alitrunk width, alitrunk length (frontal profile of the pronotum to the insertion of the petiole in lateral view), head length, and head width (behind the eyes) were measured in 54 queens. Queens and workers collected near Itabuna were compared to seven workers and two syntypes, one worker and one intermorph, of *P. villosa inversa* obtained from the British Museum (Natural History) (BMNH), London.

**Isozyme electrophoresis.** Whole ants were crushed in 50 $\mu$ l distilled water. Of the homogenate, 3 $\mu$ l were applied onto pre-soaked cellulose acetate plates, which were subsequently subjected to electrophoresis for 20-30 min at 200-230V, depending on the enzyme system, using one of three different buffer systems (0.025M Tris-glycine pH 8.6, 0.1M Tris-citrate pH 7.0 and pH 8.2, 0.1M Tris-maleate-EDTA pH 7.4 and pH 8.3). Using slightly modified protocols from MURPHY et al. (1990), 25 enzymes were stained. Esterases (EST), glucose-6-phosphate isomerase (GPI), isocitrate dehydrogenase (IDH) and trehalase (TRE) were examined in greater detail. One to six workers from each of 55 colonies were investigated. For xanthine dehydrogenase (XDH) 15 colonies with one worker respectively were checked.

## Results

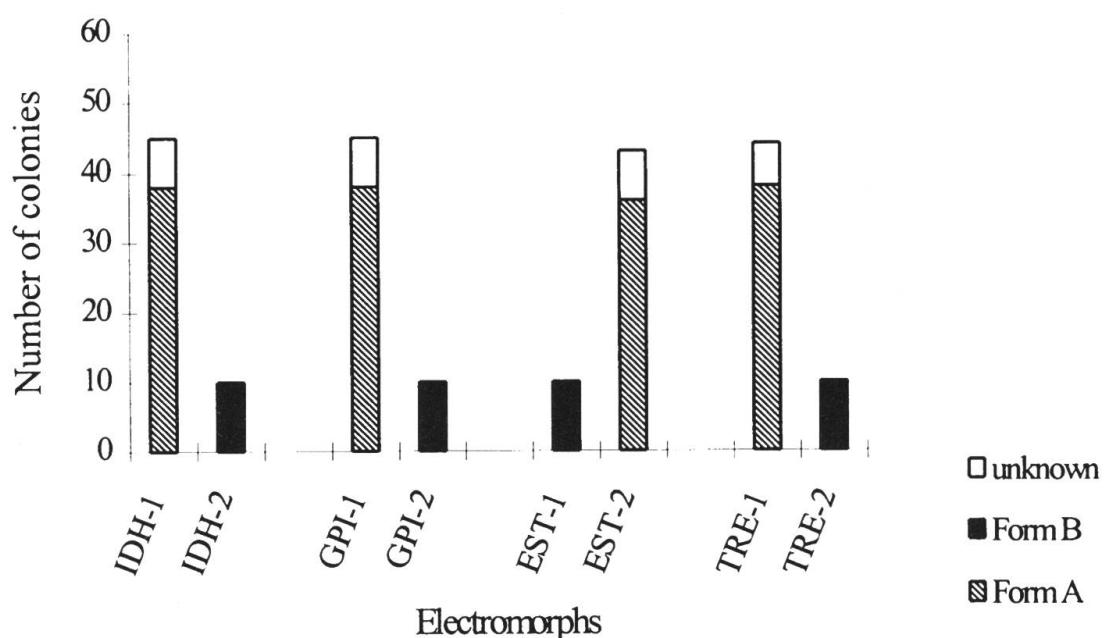
**Measurements & morphology.** All five characters measured in the 54 queens showed a significantly bimodal distribution, suggesting the existence of two morphologically distinct forms, one larger and the other significantly smaller in all examined characters (Mann-Whitney U tests,  $p<0.0001$ , see also Tab. 1). As expected, the different characters were highly correlated with each other (multiple regression  $r = 0.96$ ,  $p<0.0001$ ), hence the smaller forms appeared to be isometrically reduced. Small queens (here referred to as "Form A") were completely black, their clypeus was conspicuously elongated and their petiole was anteriorly concave. The anterior margin and the upper surface of the petiole formed a sharp angle in lateral view. In large queens ("Form B"), petiole, parts of the legs, and the base of the gaster were of dark reddish coloration. Their clypeus was comparatively short. The anterior margin of the petiole was not concave and formed a right angle with the upper surface. Dissection of the queens' ovaries revealed that they consist of 2 x 3 ovarioles in Form A queens and of 2 x 4 ovarioles in Form B queens.

Of the two syntypes of the BMNH, the worker clearly belonged to Form A, the intermorph to Form B. Six of the seven workers of *Pachycondyla villosa inversa* in the BMNH, all from Guyana, resembled Form B in morphology. Three of them were labeled "*Neoponera* (s.str.) *villosa* (subsp. *inversa* ?)". One, labeled "*Neoponera* ? *villosa* subsp. *inversa*" from Rio de Janeiro, could not be assigned, because its clypeus was more elongated than in the other forms.

**Isozyme electrophoresis.** Twenty of a total of 25 examined enzyme systems could be reliably stained. The banding patterns of four enzymes (EST, GPI, IDH, TRE) showed clear differences between individuals from 38 colonies of Form A and 10 colonies of Form B (Fig. 1). XDH showed differences between the two forms in all 15 examined colonies. Both forms were fixed for different electromorphs at all five loci, which therefore can be used as diagnostic characters. Banding patterns of malate dehydrogenase (MDH), phosphate dependent malate dehydrogenase (MDHP), phosphoglucomutase (PGM) and phosphogluconate dehydrogenase (PGDH) were probably polymorphic, but could not be interpreted because of insufficient resolution on cellulose acetate plates.

Variation in morphological characters of individuals from seven colonies made it impossible to assign them unambiguously to forms A or B, respectively. However, using isozyme electrophoresis we could clarify their status: their banding patterns were always identical to those of Form A (Fig.1).

	Form A		Form B	
	Mean	SD	mean	SD
Alitrunk length	4.553	0.130	5.396	0.134
Alitrunk width	2.099	0.075	2.412	0.072
Thorax length	3.353	0.076	4.081	0.150
Head length	2.919	0.077	3.250	0.093
Head width	2.802	0.076	3.215	0.080

Tab. 1: Measurements [mm] of queens from two different forms of *Pachycondyla villosa* from Itabuna, Brazil.Fig. 1: Distribution of isozyme electromorphs in two morphologically different forms of *Pachycondyla villosa* from Itabuna, Brazil.

## Discussion

The banding patterns of five different enzyme systems clearly showed differences between two morphologically different, sympatric forms of the ant *Pachycondyla villosa*, A and B. The lack of heterozygous banding patterns in these enzymes and the fact that in approximately 100 examined colonies the two forms never co-occurred, strongly suggest that they represent two different species and not an intraspecific polymorphism. Pairs of sibling species, i.e., reproductively isolated taxa which are difficult to distinguish by morphological analysis, are quite common in ants. They often are only detected by detailed morphometric studies of large numbers of individuals or by molecular methods (e.g. WARD, 1980). An unambiguous distinction between the two species studied in our investigation is probably also only possible by a more detailed morphological investigation or isozyme electrophoresis: on a first glance, individuals from a small

minority of colonies were morphologically more or less intermediate between forms A and B. However, in all five diagnostic isozyme loci, the banding patterns of workers from these colonies were identical to those of "Form A".

The taxonomic status of the two species is not yet clear. Most early references do not record the morphology of the described ants in sufficient detail to allow the assignment of our forms to one of the previously described taxa (e.g. SMITH, 1858, GUÉRIN-MÉNEVILLE, 1844). ROGER (1861) reported on two forms of *Pachycondyla villosa*: one, collected in Columbia, resembles our Form A, the other, from Mexico and Demarara, resembles Form B. From individuals with intermediate morphology he excluded that the two forms are separate species. Unfortunately, ROGER did not compare his findings to *Pachycondyla villosa inversa* (then *Ponera inversa*), which he described as "species incertae sedis". According to later descriptions of the morphology of *Pachycondyla villosa inversa* (referred to as *Ponera inversa* SMITH, 1858 and *Pachycondyla villosa* var. *curvinodis*, FOREL, 1899), Form A might be identical to this taxon. Nominal *P. villosa* appears to be morphologically similar to Form B (WHEELER, 1908, GALLARDO, 1918).

A comparison with material from the BMNH, including two syntypes of *P. villosa inversa* did not clarify the taxonomic status of the Forms A and B, as it contained individuals from both.

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