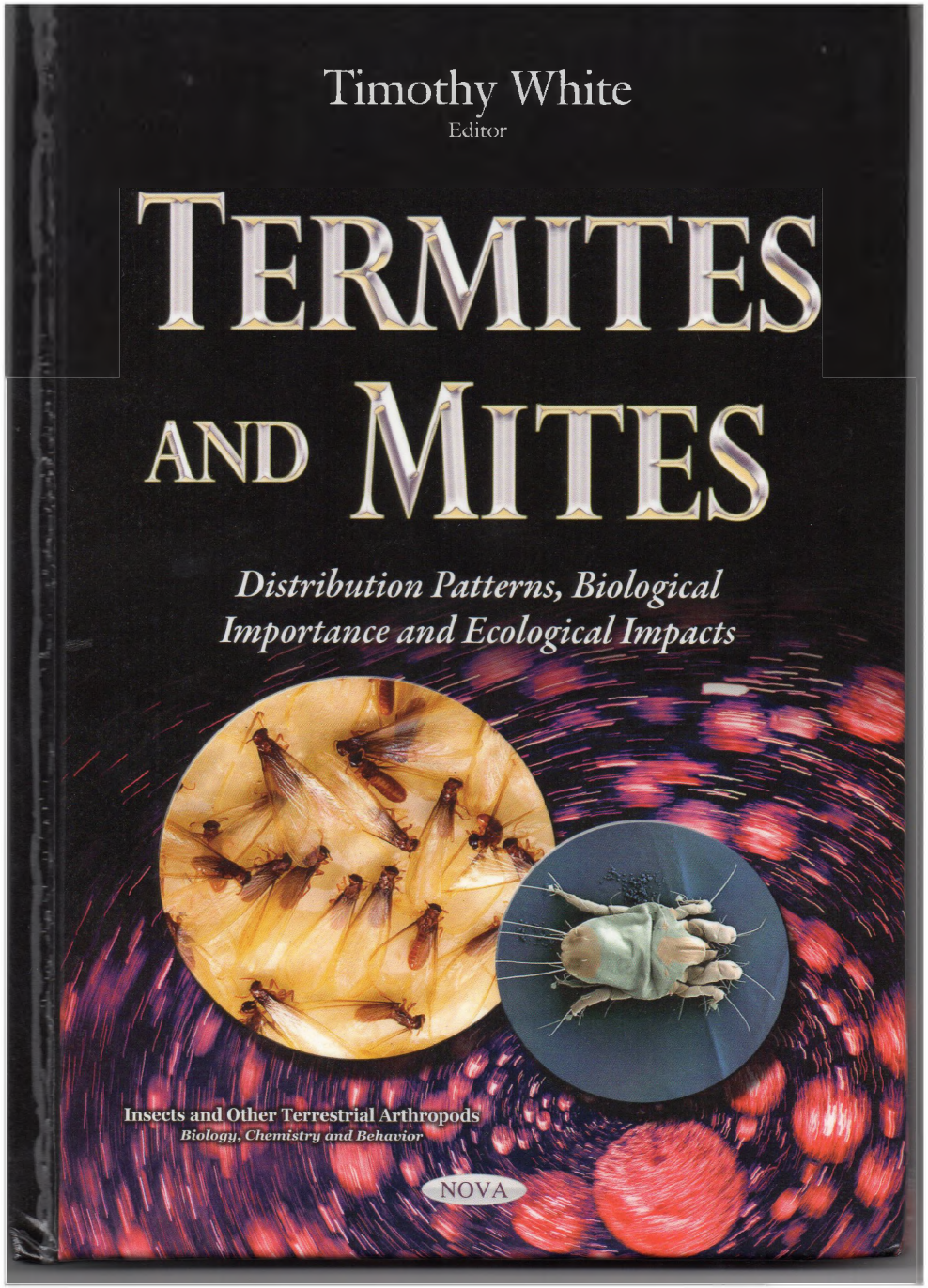

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Chapter 2

SOCIAL POLYMORPHISM AND GUT DESING IN "HIGHER TERMITES": A CASE STUDY IN NEOTROPICAL TERMITINAE

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ABSTRACT

The main structure and detailed characteristics of the digestive tube of termites are known for many genera from the Neotropical and other biogeographical regions, due to its functional, taxonomic and phylogenetic relevance. However, there are only some studies on the intraspecific intestinal differences derived from the social polymorphism and their relationships with the nutritional ecology of each species. Within higher termites (Termitidae), the Termitinae subfamily is probably paraphyletic and comprises various genera of uncertain affinities with different nesting and nutritional habits, whose soldiers have symmetric or asymmetric mandibles. In this chapter, the functional morphology of the digestive tube of ten species belonging to seven genera of neotropical Termitinae was analyzed, with the aim of characterizing and comparing the configuration of individuals from different castes. Also, the relationships among the observed structures and their ecological significance in relation to the substrates consumed by each species and the feeding mode of each caste were analyzed.

The study of workers, soldiers and winged alates included the coiling configuration, the segment structures and their internal chitinous ornamentations. Sixty five intestinal characters were analyzed and distinctive characteristics of the digestive tube of *Amitermes*, *Dihoplotermes*, *Microcerotermes*, *Neocapritermes*, *Onkotermes*, *Spinitermes* and *Termes* are given, with special attention to features of possible ecological significance. The effect of social polymorphism was evidenced in the intestinal morpho-anatomy of the analyzed Termitinae, with notable differences between the castes of each species. The gut coiling, morphology of the digestive organs and proctodeal development showed major similarities between workers and soldiers. Workers had the most developed and differentiated gut in eight of the studied species. The proctodeum occupied a higher proportion of the total gut length in workers and soldiers, while the foregut was longer in alates. With regard to the internal ornamentations, the development and sclerotization degree of the gizzard was higher in winged reproductives, which also had cuticular productions in the third proctodeal segment. Possible explanations are given for the analyzed species. However, these relationships explain only to some extent the observed intestinal morphological variations since intestinal design are certainly influenced by multiple factors.

INTRODUCTION

One of the phenomena that decisively influence the sociality and nutritional ecology of termites is the differential feeding mode of individuals from different castes within colonies. The workers provide allo-parental care to other nestmates, such as trophallaxis that supply partially processed feeding substrates. Soldiers receive solid or liquid food, while the larvae, nymphs and reproductives obtain in most cases saliva or a mixture of saliva and regurgitated food (Noirot 1995). It has been suggested that such nutritional differences between colony members could involve structural differences in the intestinal tube as well as in the absorption and digestion processes (Noirot and Noirot-Timothée 1969).

The main structure and detailed characteristics of the digestive tube of termite workers are known for many genera from the Neotropical and other biogeographical regions, due to its functional, taxonomic and phylogenetic relevance (see reviews in Fontes 1987, 1992, 1995; Noirot 2001, Sands 1992, 1998; Donovan et al. 2000, Bitsch and Noirot 2002, Bignell 2011). For workers, the intestinal anatomy provides useful characters at the generic level

and the enteric valve armature development offer valuable information at the species level. However, there are only few references about the intraspecific intestinal differences derived from the social polymorphism and their relationships with the nutritional ecology of each species. In no-Termitidae, the differences in the configuration of the digestive tube between castes of the same species appear to be rare, except for a few Rhinotermitidae soldiers where the remarkable development of the frontal gland reduces the volume of the intestine (Noirot 1995, Godoy and Torales 1996, Barsotti and Costa-Leonardo 2000, Godoy 2004). In Termitidae, the differences seem more frequent. For example, Miller (1991) reports some organs with lower diameters and lengths in soldiers and alates with respect to workers of the same species. Also, differences in the proventriculus and enteric valve internal ornamentations were detected in some species (Noirot and Noirot-Timothée 1969, Noirot 1995, 2001). At least in one Australian genus, the enteric valve armature of workers and alates are totally different (Miller 1991).

Another particular aspect of the nutritional ecology of termites are the obligated nutritional mutualisms with microorganisms belonging to the Archaea, Eubacteria and Eucarya domains, which are hosted in their intestinal tubes being part of complex communities or microbial consortia (Bignell 2000, Eggleton 2006). Population densities higher than 10^{11} cells/mL have been detected, which are of extraordinary morphological diversity, especially in the hindgut (Ohkuma and Brune 2011). The gut of termites is a rich and innovative source of microbial diversity; including some lineages known so far only for these insects and whose analysis has been recently increase by its biotechnological potential (Brune 1998, 2014; Bignell 2000, Kohler et al. 2012, Grieco et al. 2013, Santana et al. 2015). Among the most abundant prokaryotes detected so far, are methanogens, spirochetes, lactobacilli, bacteroides and Staphylococcus, while among eukaryotic organisms, flagellates, amoebas and gregarines are found (Breznak 2000, Inoue et al. 2000, Hongoh et al. 2003, König et al. 2006, Hongoh 2011). The bacterial composition differs between species and even between different organs of the digestive tube and is related to the diet of the termite (Brauman et al. 2001, Brune 2014). It has also been verified the existence of coevolution processes of host termites with their symbionts, which are vertically transferred by proctodeal trophallaxis (Hongoh et al. 2005, Noda et al. 2007). In many cases, the exact nature of the exchanges that occur between them are still unknown. Microaerobic and anaerobic metabolic processes of microbial origin have been detected in different parts of the termite gut, mainly in the proctodeum where oxygen radial and axial gradients from the periphery to the center of the

intestinal lumen occur, as well as significant variations in pH (Bignell et al. 1980, Brune 1998, Breznak 2000). Wood-feeding and litter-feeding termites do not depend exclusively on their symbionts for the degradation of cellulose and other refractory polysaccharides, since endogenous enzymes as cellulases and others have been identified (Tokuda et al. 1997, 2012; Warnecke et al. 2007, Lo et al. 2011). Intestinal microorganisms also contribute to meet the requirements of carbon, nitrogen and energy of these insects, through processes such as nitrogen fixation, acetogenesis hydrogen and carbon dioxide and uric acid degradation (Breznak 2000, Rouland and Bignell 2001, Brune and Ohkuma 2011). These symbionts also serve as a nutritional source for the termites that get high quality proteins from them (Nalepa et al. 2001, Brune 2014). The distribution of the microbiota in the intestine is not at random and many of them are attached to the surface of the intestinal walls, to internal ornamentations like spines or circular depressions or to other endosymbionts (Nakajima et al. 2005). The midgut and mixed segment communities seem less diverse and are located mainly between the peritrophic membrane and mesenteric epithelium (Bignell et al. 1980, Tokuda et al. 2000). They would come into contact with the feeding substrate through the rupture of the peritrophic by active peristalsis of the first proctodeal segment frontal end (Bignell et al. 1983). The hindgut is the location site of the most dense and diverse microorganism populations, especially the proximal end of the first and third proctodeal segments and the adjacent epithelium of the fourth proctodeal segment (Bignell 2011).

Within "higher termites" (Termitidae), the Termitinae subfamily is probably a paraphyletic assemblage that currently comprises various genera of uncertain affinities with different nesting and nutritional habits, whose soldiers have symmetric or asymmetric mandibles (Miura et al. 1998, Donovan et al. 2000, Inward et al. 2007, Bourguignon et al. 2008). The Termitinae taxonomy is not adequately resolved and some widely distributed genera as *Amitermes*, *Microcerotermes* and *Termes* need revisions (Inward et al. 2007, Bourguignon et al. 2008). For the Neotropical region, several genera like *Onkotermes*, *Cylindrotermes*, *Spinitermes*, *Orthognathotermes*, *Divinotermes* and *Genuotermes* have been recently revised or described taking into consideration along with others, the intestinal characters (Constantino et al. 2002, Rocha and Cancello 2007, 2009; Carrijo 2009, Carrijo and Cancello 2011).

In this chapter, the functional morphology of the digestive tube of ten species belonging to seven genera of neotropical Termitinae was analyzed, with the aim of characterizing and comparing its configuration among individuals from different castes.

Also, the relationships among the observed structures and their ecological significance in relation to the substrates consumed by each species and the feeding mode of each caste are discussed.

Material and Methods

The study of worker, soldier and winged alate digestive tubes included coiling or "in situ" configuration, morphology and internal chitinous ornamentations. The specimens were fixed in FAA (formalin: alcohol: acetic acid) solution and preserved in ethanol 70°. The species and castes analyzed were *Amitermes amifer* Silvestri 1901 (workers and soldiers), *Dihoplotermes inusitatus* Araujo 1961 (workers, soldiers and alates), *Microcerotermes strunckii* (Sörensen 1884) (workers, soldiers and alates), *Neocapritermes opacus* (Hagen 1858) (workers, soldiers and alates), *Onkotermes brevicorniger* (Silvestri 1901) (workers and soldiers), *Onkotermes corochus* Fontes and Torales 2008 (workers and soldiers), *Spinitermes brevicornutus* (Desneux 1904) (workers and soldiers), *Termes bolivianus* (Snyder 1926) (workers, soldiers and alates), *Termes nigritus* (Silvestri 1901) (workers and soldiers) and *Termes riograndensis* Jhering [Ihering], 1887 (workers, soldiers and alates). In species with dimorphic workers, major workers were analyzed.

Identifications were made by G. Torales and J. Coronel (UNNE) and the samples were deposited in the Isoptera (FACENAC) collection of the Facultad de Ciencias Exactas y Naturales y Agrimensura of the Universidad Nacional del Nordeste (Corrientes, Argentina). *O. brevicorniger* samples were determined by R. Constantino (UB, Brazil) and donated by J. Liotta and B. Giacosa.

The specimens were dissected and the gut exposed to observe the "in situ" configuration and the details of the segments. Sixtyfive morphoanatomic characters of the digestive tube considered by Sands (1998) were analyzed. Also, qualitative observations of other characteristics as the proventricular sclerotization degree and the internal armature of other intestinal organs (crop, stomodeal valve, first proctodeal segment, third proctodeal segment, colon and rectal valve) were analyzed. To achieve these observations, those intestinal segments were mounted in semi-permanent slides with Swan's Berlese. The characteristics of the intestinal content (condition, color) were examined by opening the third abdominal segment in individuals of each caste and species.

Measurements of the total length, segments and organs of the gut were registered with a reticule attached to a stereomicroscope, in series of 30

individuals of each species and caste from five different colonies. The number of *D. inusitatus* alates analyzed was lower (five) as well as soldiers of *O. brevicorniger* and *O. corochus* (two). The internal armature structures (ridges, spines, scales) were measured with an ocular micrometer attached to a binocular microscope (10x and 40x objectives). Thirty measures of each structure were recorded in five individuals of each species and caste. Interindividual variations are recognized in the volume and shape of the gut segments mainly due to the amount of consumed food and the contraction or relaxation of fixed insects (Fontes 1987 b, Sands 1998). Because of this, the observation and measuring of a large number of termites could characterize adequately the intestinal anatomy of each species by obtaining mean values and variation ranges for each organ. In the case of the crop, fully fed individuals were selected for measurement with maximum dilatation of this organ. As the gut segments are constituted by fragile tissues which can be distorted during observation and mounting, only the statistically significant differences between the mean values of individuals of each caste, obtained through the mean difference for independent samples t test, with $\alpha = 0.05$ were considered in the comparisons.

The terminology followed Noirot and Noirot-Timothée (1969), Sands (1972, 1998), Fontes (1987 a, b) and Noirot (1995, 2001). The G/A and C/H indexes were calculated for the worker proventriculus of all species, which refer to the development of the internal armature (Fontes 1987 b, Noirot 2001). For the attachment sites of the Malpighian tubules, the patterns established by Noirot (2001) were considered. The system of Sands (1972, 1998) was adopted for numbering and describing the proventicular ridges of *D. inusitatus* (for size differences between ridges) and the enteric valve armature of all species, as it allows to interpret the homologies in genera with significant modifications and reflects the symmetry, sclerotization and ornamentation variations observed (Donovan et al. 2000). The schemes of gut coiling and details of the segments of the digestive tube were made with a camera lucida attached to a stereomicroscope. The internal ornamentations were photographed with a digital camera attached to a binocular microscope.

Results and Discussion

The configuration of the termite gut differs according to the considered taxonomic group. In general, non-Termitidae have a relatively simple alimentary canal while in Termitidae, the worker gut is longer and shows more

structural and physiological differentiation (Noirot and Noirot-Timothée 19(Fontes 1987 b, Noirot 1992, 1995, 2001; Bignell 1994, 2000, 2011). Su complexity is evident in the species of the Termitinae subfamily analyzed this chapter, since 48 of the 65 morphoanatomic characters presented in Tal 1 showed interspecific differences and 12 of them also showed variatio between individuals of different castes.

On the bases on the considered characters, the digestive tube of t genera, species and castes considered was characterized. The interspecific a intraspecific similarities and differences with other termites and their possil relationships with diet and the feeding mode of each caste were discussed.

Coiling or *"In Situ"* Disposition of the Gut

The gut coiling and the organ structures were more similar betwe workers and soldiers of the analyzed species than with alates (Figures 1–6 According to Noirot and Noirot-Timothée (1969), the digestive tube workers and soldiers (neuters) follows a similar "plan" in Termitidae, althou the development and differentiation is generally higher in workers. Throu the comparison of both castes in the species analyzed in this chapter, it w found that the measures were higher in workers with the exception of *opacus* and *S. trispinosus* soldiers where some of the segments (crop, midg mixed segment, first and fourth proctodeal segments and rectum) and the to length of the digestive tube exceeded those of workers. The observ similarity between workers and soldiers in their gut characteristics could attributed to the fact that the soldiers of these species receive solid food, w little alteration from the raw nutrients consumed by workers. In the cas where significant differences in the intestinal anatomy between individuals both castes were reported, the soldiers receive liquid food, such as in t genera *Odontotermes*, *Pericapritermes* and *Paracapritermes* (Noirot a Noirot-Timothée 1969, Miller 1991).

The digestive tube of the alates analyzed in this chapter show differences in development and coiling with respect to workers and soldie which are described below. Afterwards, in female primary reproductives, t coiling, morphology and development of the intestinal segments suf significant alterations due to physogastry, mainly involving lengthening a widening of the midgut and hindgut.

	amifer					*brevicorniger*		*corochus*								*trispinosus*		*bolivianus*			*nigritus*		*riograndensi*		
	w	s	w	s	a	w	s	w	s	w	s	a	w	s	a	w	s	w	s	a	w	s	w	s	a
(57)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
(58)	1	1	1	1	1	1	1	1	1	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1
(59)	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
(60)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
(61)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
(62)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
(63)	3	3	1	1	1	2	2	2	2	1	1	1	1	1	1	2	2	3	3	1	2	2	2	2	1
(64)	3	3	3	3	3	4	4	4	4	4	4	3	3	3	3	4	4	3	3	3	3	3	3	3	3
(65)	4	4	4	4	3	3	3	3	3	2	2	2	2	2	2	3	3	3	3	3	3	3	3	3	3
0 (66)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1 (67)	2	2	4	4	4	3	3	3	3	2	2	2	4	4	4	2	2	2	2	2	2	2	2	2	2
2 (68)	2	2	2	2	2	2	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3 (69)	2	2	1	1	1	1	1	1	1	2	2	1	1	1	1	2	2	2	2	2	2	2	2	2	2
4 (70)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5 (71)	1	1	1	1	1	1	1	1	1	3	3	3	2	2	2	3	3	3	3	3	3	3	3	3	3
6 (72)	3	3	3	3	3	3	3	3	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
7 (73)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8 (74)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9 (75)	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
0 (76)	2	2	4	4	4	4	4	4	4	6	6	4	6	6	4	6	6	6	6	4	6	6	6	6	4
1 (77)	2	2	2	2	3	1	1	1	1	2	3	3	1	1	3	2	2	2	2	2	1	1	2	1	2
2 (78)	1	1	4	4	1	3	3	3	3	1	1	1	3	3	1	1	1	3	3	1	3	3	3	3	1

	A. amifer		*M. strunckii*			*O. brevicorniger*		*O. corochus*		*D. inusitatus*			*N. opacus*			*S. trispinosus*		*T. bolivianus*			*T. nigritus*		*T. riograndei*	
	w	s	w	s	a	w	s	w	s	w	s	a	w	s	a	w	s	w	s	a	w	s	w	s
23 (79)	3	3	4	4	4	3	3	3	3	2	2	2	2	2	2	1	1	3	3	3	3	3	3	3
24 (80)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
25 (81)	2	2	1	1	1	1	1	1	1	2	2	1	1	1	1	2	2	2	2	1	2	2	2	2
26 (82)	1	1	1	1	1	2	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
27 (83)	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
28 (84)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
29 (85)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
30 (86)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
31 (87)	2	2	2	2	1	6	6	6	6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
32 (88)	2	2	3	3	3	4	4	4	4	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2
33 (89)	2	2	3	3	3	4	4	4	4	1	1	1	3	3	3	2	2	2	2	2	2	2	2	2
34 (90)	2	2	3	3	3	4	4	4	4	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2
35 (91)	2	2	3	3	3	4	4	4	4	1	1	1	3	3	3	2	2	2	2	2	2	2	2	2
36 (92)	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
37 (93)	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
38 (94)	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
39 (95)	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
40 (96)	4	4	3	3	3	1	1	1	1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
41 (97)	4	4	4	4	4	1	1	1	1	3	3	3	4	4	4	4	4	4	4	4	4	4	4	4
42 (98)	4	4	4	4	4	1	1	1	1	3	3	3	4	4	4	4	4	4	4	4	4	4	4	4
43 (99)	4	4	4	4	4	1	1	1	1	3	3	3	4	4	4	4	4	4	4	4	4	4	4	4
44 (100)	3	3	2	2	2	D	D	D	D	4	4	4	4	4	4	1	1	4	3	4	3	3	3	3
45 (101)	3	3	2	2	2	D	D	D	D	3	3	3	4	4	4	1	1	2	2	3	2	2	2	2
46 (102)	3	3	1	1	1	D	D	D	D	4	4	4	4	4	4	1	1	4	3	4	3	3	3	3

Table 1. (Continued)

	A. amifer		*M. strunckii*			*O. brevicorniger*		*O. corochus*		*D. inusitatus*			*N. opacus*			*S. trispinosus*		*T. bolivianus*			*T. nigritus*		*T. riograndensis*		
	w	s	w	s	a	w	s	w	s	w	s	a	w	s	a	w	s	w	s	a	w	s	w	s	a
47 (103)	3	3	1	1	1	D	D	D	D	3	3	3	4	4	4	1	1	2	2	3	2	2	2	2	2
48 (104)	2	2	2	2	2	D	D	D	D	2	2	2	2	2	2	2	2	1	1	1	1	1	1	1	1
49 (105)	2	2	2	2	2	D	D	D	D	2	2	2	2	2	2	2	2	1	1	1	1	1	1	1	1
50 (106)	2	2	2	2	2	D	D	D	D	2	2	2	2	2	2	2	2	1	1	1	1	1	1	1	1
51 (107)	2	2	2	2	2	D	D	D	D	2	2	2	2	2	2	2	2	1	1	1	1	1	1	1	1
52 (108)	1	1	1	1	1	D	D	D	D	1	1	1	2	2	2	2	2	1	1	1	1	1	1	1	1
53 (109)	1	1	2	2	2	D	D	D	D	1	1	1	2	2	2	2	2	1	1	1	1	1	1	1	1
54 (110)	1	1	1	1	1	D	D	D	D	1	1	1	2	2	2	2	2	1	1	1	1	1	1	1	1
55 (111)	1	1	2	2	2	D	D	D	D	1	1	1	2	2	2	2	2	1	1	1	1	1	1	1	1
56 (112)	1	1	1	1	1	D	D	D	D	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
57 (113)	1	1	1	1	1	D	D	D	D	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
58 (114)	1	1	1	1	1	D	D	D	D	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
59 (115)	1	1	1	1	1	D	D	D	D	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
60 (116)	1	1	1	1	1	D	D	D	D	1	1	1	2	2	2	2	2	1	1	1	1	1	1	1	1
61 (117)	2	2	1	1	1	D	D	D	D	2	2	2	3	3	3	1	1	3	3	3	3	3	3	3	3
62 (118)	1	1	3	3	3	D	D	D	D	1	1	1	2	2	2	1	1	1	1	1	1	1	1	1	1
63 (119)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
64 (120)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
65 (121)	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D

The numbers of the characters in Sands (1998) are indicated in parentheses. References: w: workers, s: soldiers, a: alates, D: dependent characters.

However, some features of taxonomic importance as the mixed segment structure were similar to those of the neuters in almost all species, as recognized in other Termitidae (Noirot and Noirot-Timothée 1969, Moraes et al. 1990, Miller 1991, Godoy and Torales 1999 a).

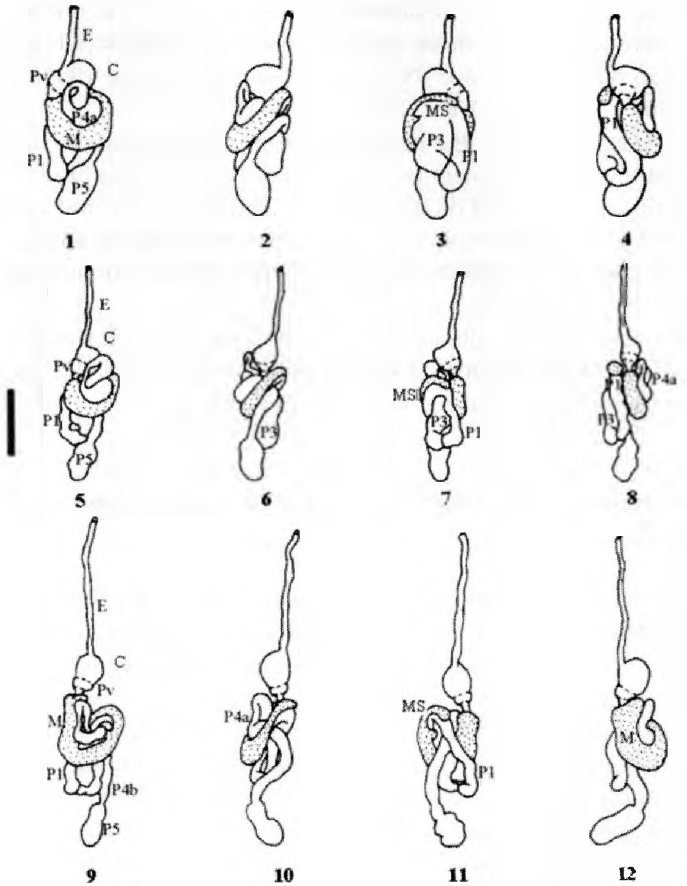
The relative position of the beginning of the midgut-hindgut junction and of the end of the mixed segment were different between castes in *D. inusitatus*. In neuters, this union started on the left of the abdomen in ventral view and its posterior end rear the right side (Figures 3, 7), while in alates it started in the right of the abdomen in dorsal view and ended in the left in ventral view (Figure 11).

The union of the Malpighian tubules to the intestinal wall was visible only in alates of four of the considered species (Figures 11, 23, 35, 47, 58) since in their respective neuters it was hidden by the gut coiling.

Measurements and Proportions of the Gut Segments and Regions

The proportion of total gut length represented by the stomodeum was higher in alates than in workers and soldiers, due to the remarkable length of the esophagus by the longer thorax in winged individuals of this caste. In alates of other neotropical Termitidae similar (*C. cumulans* and *T. riograndensis*) or smaller (*C. fulviceps*) percentages have been registered (Godoy and Torales 1999 a). The hindgut accounted for the largest intestinal proportion in the sterile castes. The percentages of worker proctodeal lengths were significantly higher than those of soldiers in the genera *Neocapritermes* and *Termes*. The individuals of the three castes of *T. riograndensis* showed the lowest percentages of the hindgut length recorded in this study, in correspondence with the data previously reported for this species (Godoy and Torales 1993).

Some of the organs were shorter in alates than in workers and soldiers, as the crop (with the exception of *D. inusitatus*) and the third proctodeal segment. The proportional length of the anterior portion of the midgut with respect to the mixed segment was another character that recorded differences between neuters and alates for the two species of *Termes*, where the midgut was shorter or almost equal to the mixed segment in neuters, but longer than this segment in *T. bolivianus* and *T. riograndensis* alates. In *N. opacus* and *Onkotermes*, the midgut formed a short mesenteric arc in neuters, as previously reported for the last genus (Constantino et al. 2002, Torales and Fontes 2008).



References: E: esophagus, C: crop, Pv: proventriculus or gizzard, M: midgut, MS: mixed segment, P1: first proctodeal segment, P3: third proctodeal segment, P4a: fourth proctodeal segment or proximal colon, P4b: distal colon, P5: rectum.

Figures 1-12. Coiling of the gut in *D. inusitatus* workers (1-4), soldiers (5-8) and alates (9-12). 1,5,9: dorsal view; 2,6,10: right view; 3, 7, 11: ventral view; 4, 8, 12: left view.

Digestive Tube Structure and Morphology

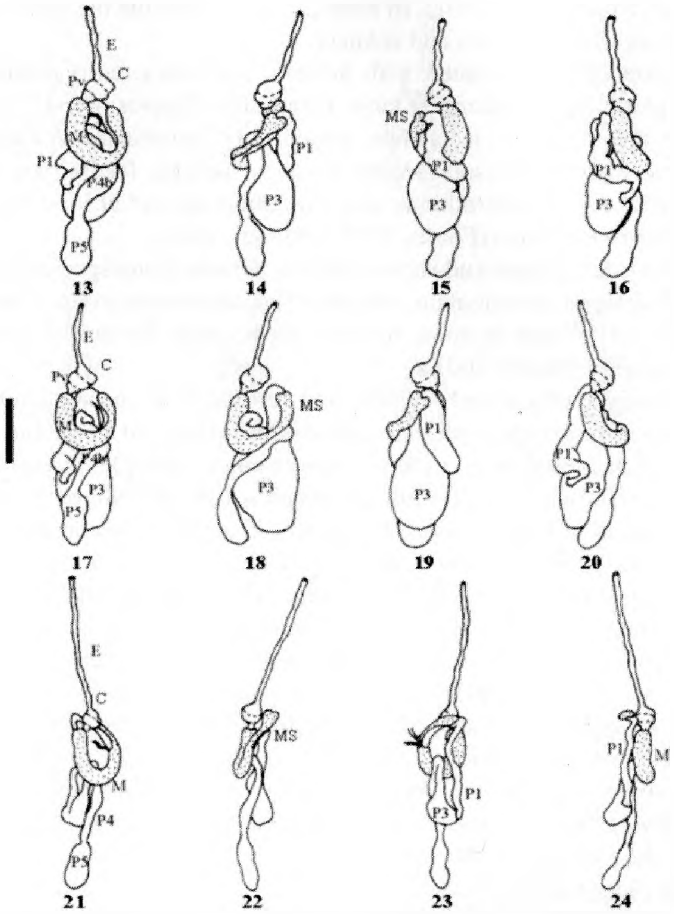
The structure and location of the esophagus, tubular mediodorsal or slightly to the left at the anterior abdominal end, as observed in the studied species, are similar in almost all termites in both Termitidae and non

Termitidae (Noirot 1995, 2001). In alates, it was noticeable the major length of this organ regarding workers and soldiers.

The crop normally dilated with bilateral symmetry, as registered in the analyzed group, is recognized in most Termitidae (Figures 61-64). This organ is widely dilated only in a few genera of Termitinae (*Euchilotermes*, *Ophiotermes*) and other soil-feeding termites, species feeding on degraded plant material in Nasutitermitinae and Syntermitinae and at least one forager genus (*Constrictotermes*) (Fontes 1987 b, Miller 1991).

The proximal midgut end showed, in the *Termes* group species analyzed, a variable developed diverticulum, absent in the *Amitermes* group. One or more diverticula are present in some termites from other Termitinae groups and other subfamilies (Noirot 2001).

The presence of a mixed segment was verified in all the studied taxa as in most Termitidae, except subfamilies Macrotermitinae and Foraminitermitinae and few Apicotermitinae genera (*Apicotermes* group), Nasutitermitinae (*Subulitermes* group) and Termitinae (Fontes 1987 b, Noirot 1995, 2001). There are differing hypotheses about the origin of this segment, as it has been proposed their monophyly in Termitidae (Fontes 1987 b) or its independent appearance in at least two groups of this family (Noirot 2001, Bitsch and Noirot 2002). While the absence of the mixed segment is considered plesiomorphic in non-Termitidae, Macrotermitinae and Foraminitermitinae, the most accepted hypothesis for the *Subulitermes* group is a regressive evolution. In Apicotermitinae instead, the hypothesis of the regression would not be the most parsimonious (Noirot 2001). Two of the Termitinae genera lacking mixed segment are *Procapritermes* and *Pseudomicrotermes* with unclear phylogenetic relationships because of their intestinal characters as it could not be established whether that absence is a basal characteristic or a regression (Sands 1998, Donovan et al. 2000). The mixed segment constituted by a single extension of the midgut external to the intestinal curvature, as observed in all the analyzed Termitinae appears in the Nasutitermitinae (*Nasutitermes* and *Subulitermes* groups) and Termitinae (*Cubitermes*, *Termes*, *Amitermes* and *Pericapritermes* groups). Within the polyphyletic hypothesis of the mixed segment origin, it is considered that this type have arisen once, giving rise to a clade including these six groups (Noirot 2001, Bitsch and Noirot 2002). The mesenteric prolongation of the mixed segment do not completely surround the gut lumen at any point in almost all Termitidae. In Termitinae, it surrounds completely the intestinal lumen only the genus *Promirotermes* (Sands 1998, Noirot 2001).



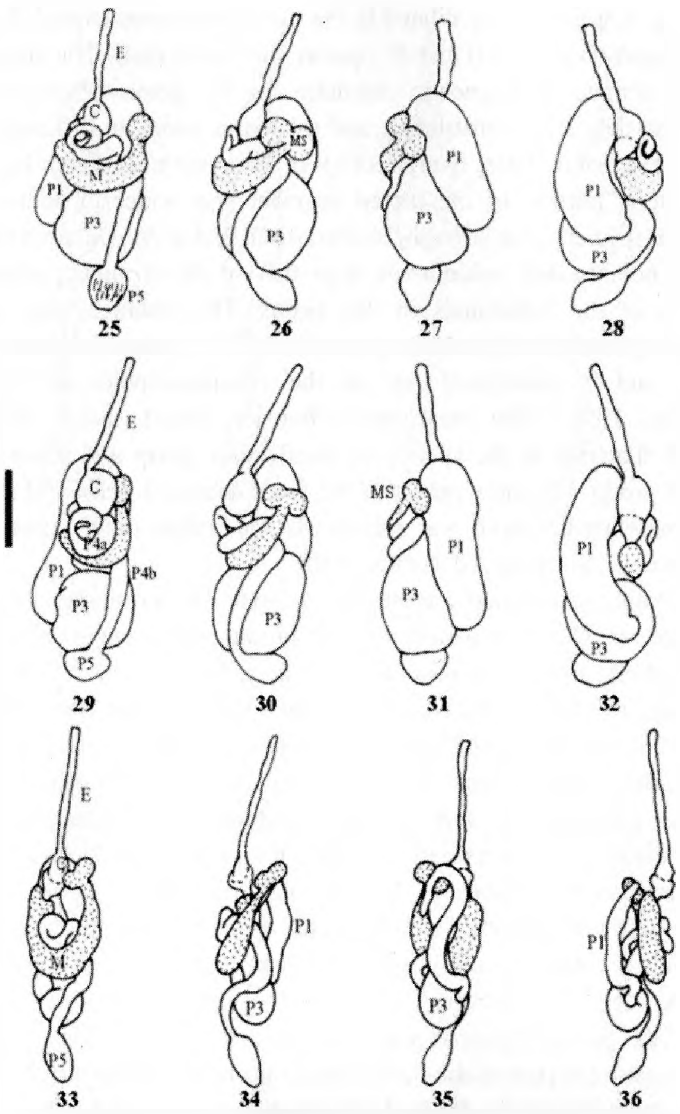
References: E: esophagus, C: crop, Pv: proventriculus or gizzard, M: midgut, MS: mixed segment, P1: first proctodeal segment, P3: third proctodeal segment, P4a: fourth proctodeal segment or proximal colon, P4b: distal colon, P5: rectum.

Figures 13-24. Coiling of the gut in *M. strunckii* workers (13-16), soldiers (17-20) and alates (21-24). 13, 17, 21: dorsal view; 14, 18, 22: right view; 15, 19, 23: ventral view; 16, 20, 24: left view.

The mixed segment configuration showed particular characteristics in each genus, regarding the dilatation and the relative length of the mesenteric portion. The mesenteric extension showed weak dilatation and rounded distal end in *A. amifer*, *D. inusitatus*, *S. trispinosus* and the three *Termes* species. On the contrary, it was strongly dilated in the two *Onkotermes* (rounded end), *M. strunckii* (heart-shaped end) and *N. opacus* (bilobated end). The shape of the mesenteric tongue is diagnostic character for the genus *Microcerotermes*, although the degree of constriction and dilatation varies in different species (Kovoor 1959, Sands 1998, Noirot 2001). In *M. strunckii* (Figures 14, 18, 22), the mesenteric portion of the mixed segment was wider in alates than in workers and soldiers. The strongly bilobed distal end in *N. opacus* (Figures 26, 30), most notable and voluminous than that of *M. strunckii*, allowed the recognition of the individuals in this genus. This characteristic has been previously reported for other *Neocapritermes* (*N. araguaia*, *N. braziliensis* and *N. talpa*) and is considered one of the synapomorphies of this genus (Constantino 1998). The mesenteric extension ended shortly before the proctodeal dilatation in the species of the *Termes* group and reach it in the *Amitermes* group. For the workers of the three analyzed *Termes* (Figures 39, 51), the mesenteric tongue was weakly dilated unlike other species of the genus where it is not enlarged (Sands 1998).

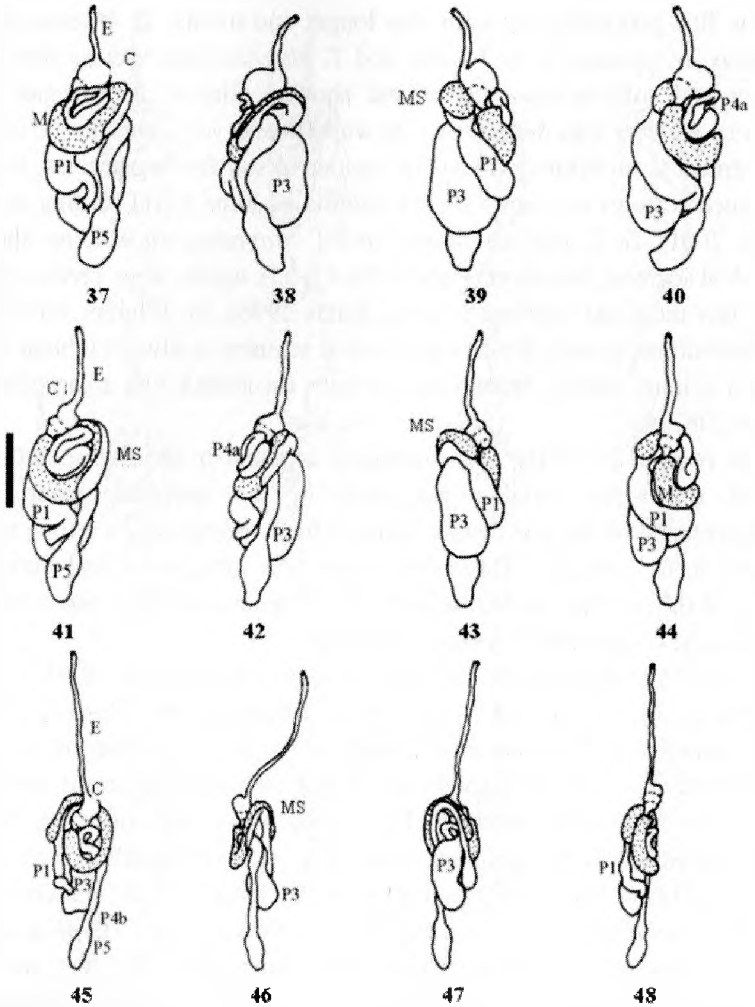
Four malpighian tubules are present in almost all Termitidae (two tubules in a few species). They are usually tubular throughout its length, as recognized in the studied group. This number is regarded as one of the family synapomorphies (Noirot and Noirot-Timothée 1969, Noirot 2001, Bitsch and Noirot 2002). The tubules are joined at the midgut-hindgut junction, except in Apicotermitinae and extend directly from the insertion point in most Termitidae although in some Macrotermitinae, Foraminitermitinae and Nasutitermitinae genera are briefly withdrawn into the midgut (Sands 1998).

The attachment pattern of the Malpighian tubules showed different characteristics in the analyzed species. In the *Amitermes* group, the connection of the tubules is direct to the intestinal wall in pairs, each with united bases (Figures 65, 66). This pattern (2B) is also observed in the *Pericapritermes* group and the genus *Orthognathotermes* of Termitinae, the *Syntermes* group (Syntermitinae) and part of the *Subulitermes* group (Nasutitermitinae) (Noirot 2001, Rocha and Cancello 2009). In the studied species of the *Termes* group (Figures 67, 68) except *N. opacus*, a diverticulum or malpighian nodule was observed, very frequent in the 2C pattern and in some *Subulitermes* group species (Noirot 2001, Bitsch and Noirot 2002) where the tubules are attached to the intestinal wall in a unique base. This 2C pattern is only present in *Termes* and *Cubitermes* groups of Termitinae (Noirot 2001). In *N. opacus*, the malpighian nodule was not differentiated and the tubules were attached on a fold of the intestinal wall.



References: E: esophagus, C: crop, Pv: proventriculus or gizzard, M: midgut, MS: mixed segment, P1: first proctodeal segment, P3: third proctodeal segment, P4a: fourth proctodeal segment or proximal colon, P4b: distal colon, P5: rectum.

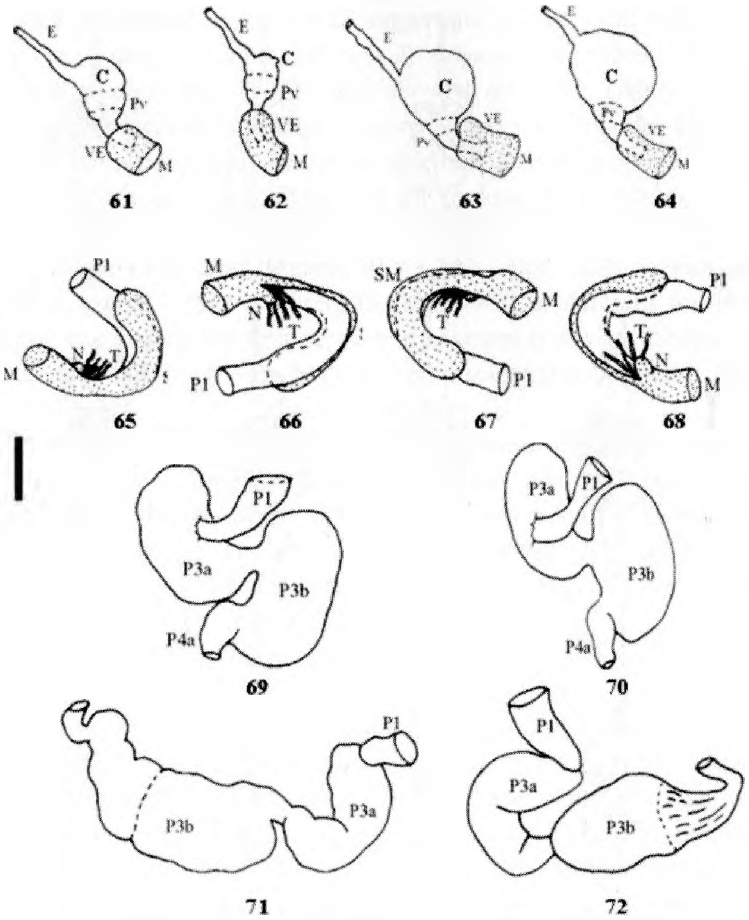
Figures 25-36. Coiling of the gut in *N. opacus* workers (25-28), soldiers (29-32) and alates (33-36). 25, 29, 33: dorsal view; 26, 30, 34: right view; 27, 31, 35: ventral view; 28, 32, 36: left view.



References: E: esophagus, C: crop, Pv: proventriculus or gizzard, M: midgut, MS: mixed segment, P1: first proctodeal segment, P3: third proctodeal segment, P4a: fourth proctodeal segment or proximal colon, P4b: distal colon, P5: rectum.

Figures 37-48. Coiling of the gut in *T. bolivianus* workers (37-40), soldiers (41-44) and alates (45-48). 37, 41, 45: dorsal view; 38, 42, 46: right view; 39, 43, 47: ventral view; 40, 44, 48: left view.

The first proctodeal segment was located on the left side of the abdomen in all the Termitinae analyzed in this chapter, although its shape, length, volume and location vary in Termitidae without clear correlations with diet (Donovan et al. 2000, Noirot 2001).



References: E: esophagus, C: crop, Pv: proventriculus or gizzard, VE: stomodeal valve, M: midgut, MS: mixed segment, N: Malpighian nodule, T: Malpighian tubules, P1: first proctodeal segment, P3a: anterior third proctodeal segment, P3b: posterior third proctodeal segment, P4a: fourth proctodeal segment or proximal colon.

Figures 61-72. Gut segments in *A. amifer* and *S. trispinosus* workers and soldiers. 61-64: foregut, 65-68: mixed segment, 69-72: third proctodeal segment or paunch. 61, 65, 69: *A. amifer* worker; 62, 66, 70: *A. amifer* soldier; 63, 67, 71: *S. trispinosus* worker; 64, 68, 72: *S. trispinosus* soldier.

Such compartmentalization has been registered for all species of *Spinitermes* (Carrijo 2009). As recorded in the observed taxa, the third proctodeal segment lacks diverticula in almost all Termitinae except in the *Cubitermes* group that have a variable diverticulum (tubular, lobed or spiral)

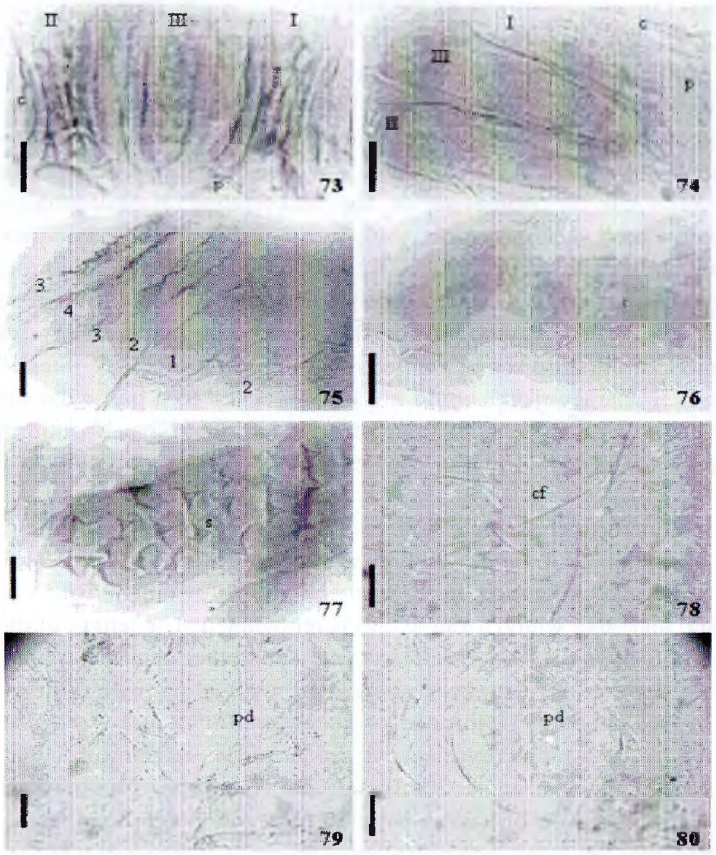
regarded as one autapomorphy of the group (Sands 1998, Donovan et al. 2000, Noirot 2001, Bitsch and Noirot 2002). The three castes of *D. inusitatus* showed lesser expansion of the third proctodeal segment than the rest of the described Termitinae. The shape of the third proctodeal segment seems correlated with diet as a single globular or pyriform compartment is recognized in wood and grass feeding species, sometimes a settlement for enteric valve can be observed. In the soil-feeding Nasutitermitinae and Termitinae, this segment is the most voluminous, complex and compartmentalized intestinal organ (Bignell and Eggleton 1995, Noirot 2001). In the Syntermitinae with mandibulate soldiers, the first and third proctodeal segments are equally important for the proctodeal hypertrophy, with the development of the third segment relatively minor in foragers (Fontes 1987 b). Other paunch characteristics could not be associated to a particular diet, like the considerable length of its anterior portion in some Nasutitermitinae (*Nasutitermes*, *Cortaritermes*) or the fewer development of its distal portion (Fontes 1987 b).

The colon formed a single coil on the species analyzed in this chapter, with the exception of *A. amifer* and *M. strunckii* workers and soldiers where it showed additional curvatures. In *M. strunckii*, the arrangement differs between neuters and alates, in which a single coil is recognized. The length of the colon is smaller in foraging Nasutitermitinae genera and other genus as *Nasutitermes* where this feature has not been associated with a particular diet (Fontes 1987b). In soil-feeding Termitinae of the *Cubitermes* and *Pericapritermes* groups and some Apicotermitinae the colon is longer (Noirot 1992, 2001; Bignell 1994, Donovan et al. 2000). For the *Amitermes* group is generally recognized the presence of a short colon (Noirot 2001) although Sands (1998) and Donovan et al. (2000) state that the colon is long in *Microcerotermes* workers, as noted in *M. strunckii*.

The characteristics of the rectum, ovoid and moderately voluminous, are uniform in Termitidae, where the six pads are less developed than in other families (Noirot 1995, 2001).

Internal Ornamentations of the Gut Segments

With regard to the internal armature of the gut segments, some studies have pointed out differences between individuals of different castes in Termitinae (Noirot and Noirot-Timothée 1969, Miller 1991), as was registered during this study (Figures 73-136).



References: I, II, III: proventricular ridges, c: columns, p: pulvilli, 1-4: enteric valve ridges, s: spines; pd: rectal pads, cf: cuticular filaments. Scale bars: 75, 79 = 0.1 mm; 73, 74, 76 = 50 µm; 77, 78, 80 = 10 µm.

Figures 73-80. Internal ornamentations of *A. amifer* workers and soldiers: 73: worker gizzard, 74: soldier gizzard, 75: worker enteric valve, 76: soldier enteric valve, 77: worker enteric valve (detail), 78: cuticular filaments in worker colon, 79: worker rectal valve, 80: worker rectal valve (pad detail).

In all the species and castes analyzed, the crop cuticle in its anterior portion showed a fine striation and the posterior region presented scales with short spines on the edges (Figure 124). This internal ornamentation of the crop is considered a plesiomorphic character shared with some Blattodea (Noirot 1995).

The stomodeal valve ornamentation, consisting of longitudinal grooves, is common to all Termitidae, Rhinotermitidae and Serritermitidae, constituting a probable synapomorphy of a clade comprising these three families (Noirot 1995, 2001).

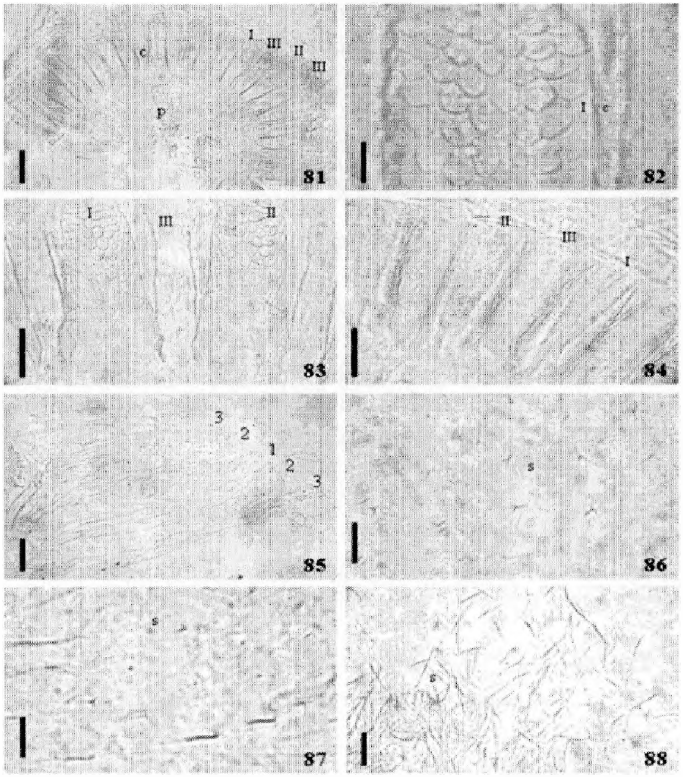
The worker proventriculus or gizzard exhibited in nine of the analyzed species a complete internal chitinous armature of the generalized type of Termitidae with 24 ridges (six of type I, six of type II and 12 of type III). In *D. inusitatus*, the proventricular armature was reduced to the pulvilar belt in workers and soldiers (Figures 92, 93) and showed a short columnar belt in alates (Figure 94), representing a distinctive character.

Thus, the genus *Dihoplotermes* is the first of the Termes group which shows a significant reduction in the proventricular armature, observed so far only in species of the *Cubitermes* group and Apicotermitinae (Noirot 2001, Bitsch and Noirot 2002). In *A. amifer*, the G/A index (Table 2) was the lowest among the analyzed species and corresponds to a small gizzard as confirmed for Nasutitermitinae species (Fontes 1987 b).

The highest values of both indexes were recorded in *M. strunckii* workers (Figures 81, 82), emphasizing the remarkable development of the gizzard ornamentation, similar to those of Syntermitinae (Fontes 1987 b, Noirot 2001).

Table 2. Proventricular indexes calculated for workers of neotropical Termitinae

Group	Species	G/A Index	C/H Index
Amitermes	*A. amifer*	1/20	0.07
	M. strunckii	1/9	0.18
	O. brevicorniger	1/13	0.12
	O. corochus	1/12	0.13
Termes	*D. inusitatus*	1/17	-
	N. opacus	1/13	0.09
	S. trispinosus	1/19	0.05
	T. bolivianus	1/14	0.07
	T. nigritus	1/15	0.07
	T. riograndensis	1/17	0.07



References: I, II, III: proventricular ridges, c: columns, p: pulvilli, 1-4: enteric valve ridges, s: spines. Scale bars: 81, 85, 88 = 0.1 mm; 82, 86, 87 = 10 µm; 83, 84 = 50 µm.

Figures 81-88. Internal ornamentations of *M. strunckii* workers, soldiers and alates. 81:worker gizzard, 82: column of worker gizzard, 83: soldier gizzard, 84: alate gizzard, 85: worker enteric valve, 86: soldier enteric valve, 87: worker enteric valve (detail), 88: spines in alate third proctodeal segment.

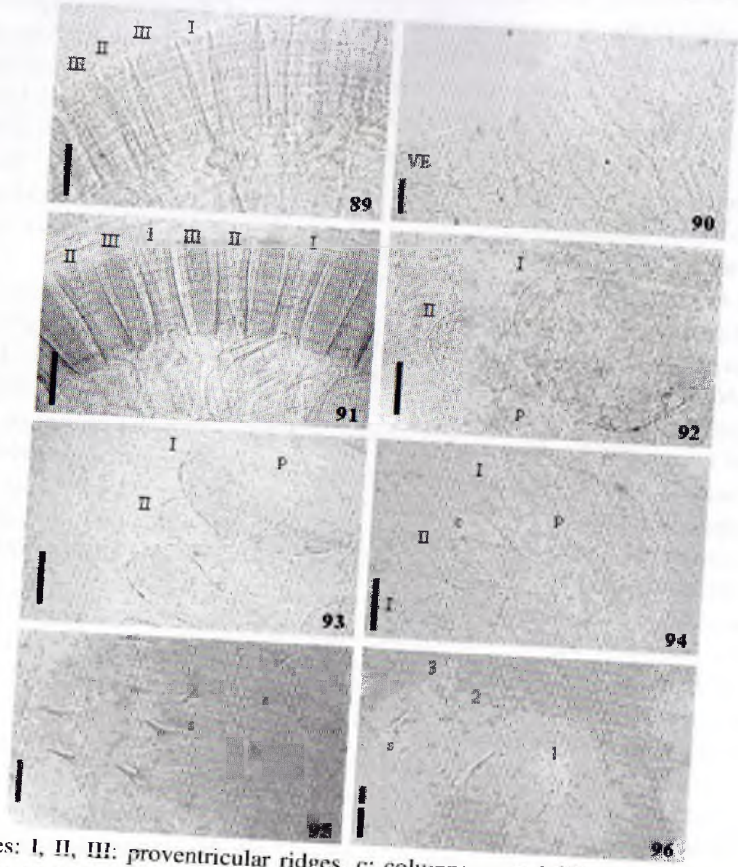
Also, the pulvilli of type I ridges were longer in relation to the columns (three to four times its length) in this species. In *S. trispinosus* the C/H ratio showed the lowest value of the analyzed group corresponding to an underdeveloped proventricular armature. Triangular spines similar to those of *Genuotermes* were recognized in the columns of I and II proventricular ridges (Figures 113, 114) (Noirot 2001, Rocha 2013).

According to the observations, the sclerotization of the gizzard ridges was more notable in alates of four of the five analyzed species than in workers and soldiers. In *D. inusitatus*, the alates were the only caste that had a developed columnar belt which was reduced in neuters, but the sclerotization was fainter than in the other species. In other Termitinae genera (*Cubitermes*, *Ophiotermes*) similar differences have been recorded between castes (Noirot and Noirot-Timothée 1969, Noirot 2001), with a more developed armature in alates that are anatomically the more generalized individuals. The columnar belt was developed in the remaining species and castes analyzed.

Comparing workers and soldiers, the gizzard chitinization degree was higher in workers or similar in both castes, but in two species (*S. trispinosus* and *T. bolivianus*), soldiers had stronger proventricular sclerotization. In the seven species where the lengths of the columns showed significant differences between castes, the workers had the highest values, with the exception of *S. trispinosus* soldiers. In the remaining characters measured, although significant differences could be observed between castes of each species, it could not be recognized any general patterns for the analyzed group. The relationships of the gizzard structure with diet are unclear, but its homoplasic variations have been correlated with the type of consumed food.

The reduction of the gizzard in workers, regarding the alates of each species, both in volume and in sclerotization is recognized in many soil-feeding genera. The well-developed ornamentation is related to the consumption of hard substrates as non-degraded wood (Kovoor 1969, Fontes 1987 b, Noirot 2001, Bignell 2011). In Termitinae, a faint sclerotization is recognized in the *Termes* group and columnar belt reduction in some genera of the *Cubitermes* group (Noirot and Noirot-Timothée 1969). However, despite sharing the same type of feeding substrates, there are variations within each taxonomic group (Noirot 2001).

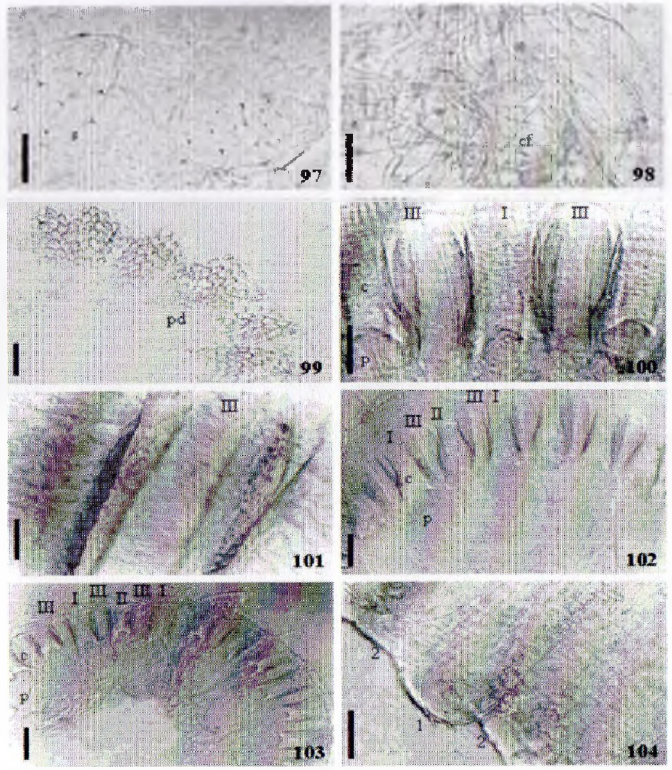
The internal armature of the enteric valve showed six unsclerotized small to medium size ridges bearing spines in seven species. In the two species of the *Onkotermes* genus the internal ornamentation was not differentiated (Figure 90), as previously reported for *O. brevicorniger* (Constantino et al. 2002). In *D. inusitatus* the ridges were partially sclerotized, with small spines (Figures 95-97). These ornamentations showed bilateral symmetry in *D. inusitatus*, tri-radial in *N. opacus* and hexa-radial in the other species. The scheme with six folds and hexa- or tri-radial symmetry is considered as the possible basal condition of Termitidae and correspond to the general structure in the *Termes* and *Amitermes* groups of the Termitinae subfamily (Noirot 2001).



References: I, II, III: proventricular ridges, c: columns, p: pulvilli, 1-4: enteric valve ridges, s: spines. Scale bars: 89, 91-94, 96 = 50 µm; 90 = 0.1 mm; 95 = 10 µm.

Figures 89-96. Internal ornamentations of *O. brevicorniger*, *O. corochus* and *D. inusitatus*. 89: *O. brevicorniger* worker gizzard, 90: *O. brevicorniger* worker enteric valve, 91: *O. corochus* worker gizzard, 92: *D. inusitatus* worker gizzard, 93: *D. inusitatus* soldier gizzard, 94: *D. inusitatus* alate gizzard, 95: *D. inusitatus* worker enteric valve, 96: *D. inusitatus* soldier enteric valve.

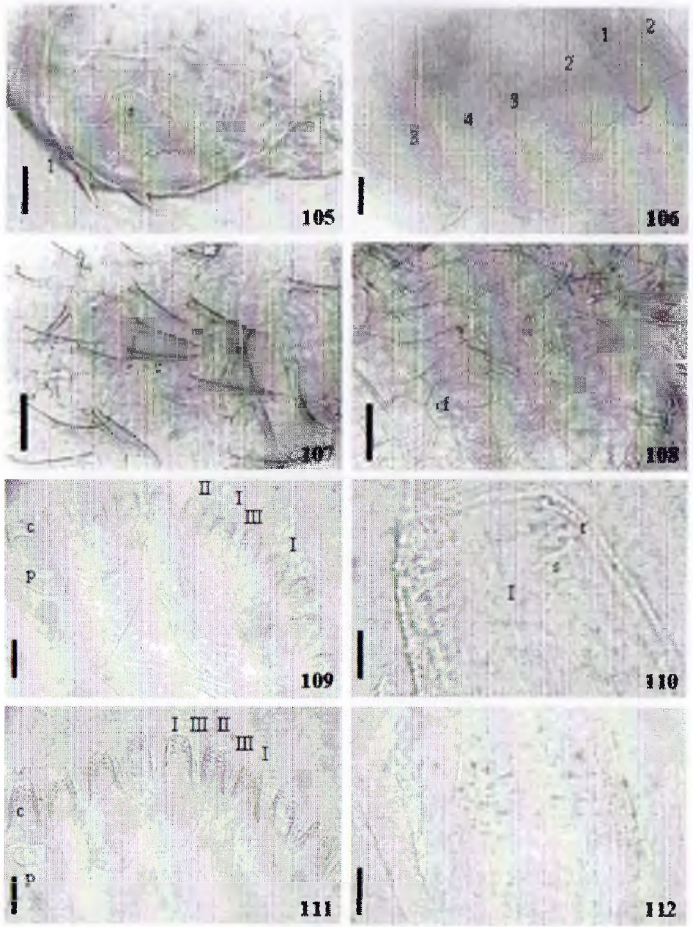
In *M. strunckii* workers, the recognition of one armature in the enteric valve with reduced ridges and spines (Figures 85-87), and also a secondary ornamentation composed by scales, allowed to add those characters to the ones previously reported for this genus that described the enteric valve as not armed (Kovoor 1959, Noirot and Noirot-Timothée 1969, Sands 1998).



References: I, II, III: proventricular ridges, c: columns, p: pulvilli, 1-4: enteric valve ridges, s: spines, cf: cuticular filaments, pd: pads. Scale bars: 97, 98, 100, 104 = 50 µm; 99, 102, 103 = 0.1 mm; 101 = 10 µm.

Figures 97-104. Internal ornamentations of *D. inusitatus* and *N. opacus*. 97: *D. inusitatus* alate enteric valve, 98: *D. inusitatus* cuticular filaments in alate third proctodeal segment, 99: *D. inusitatus* worker rectal valve, 100: *N. opacus* worker gizzard, 101: *N. opacus* worker gizzard (detail of III ridge), 102: *N. opacus* soldier gizzard, 103: *N. opacus* alate gizzard, 104: *N. opacus* worker enteric valve.

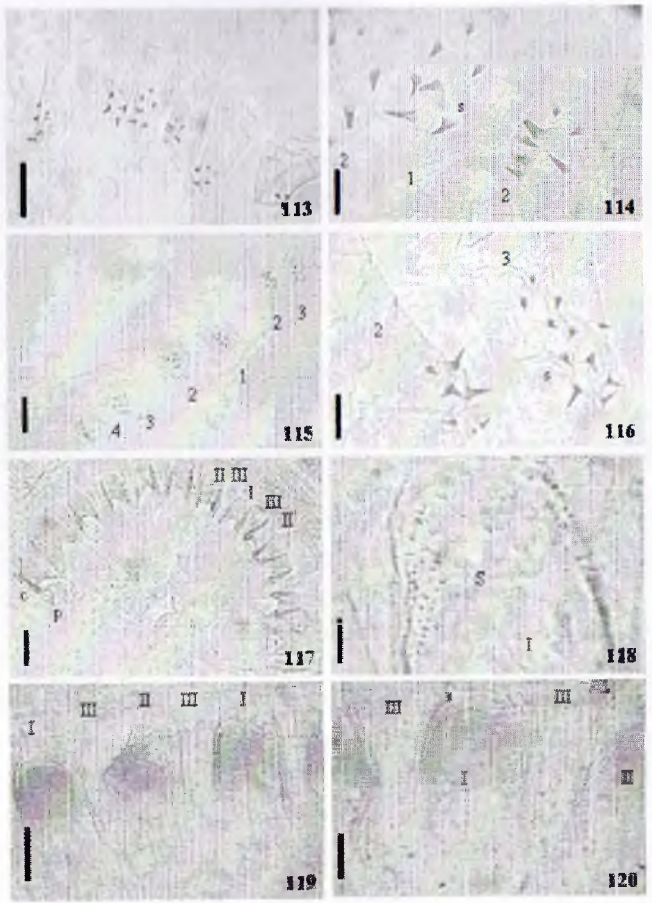
The bilateral symmetry of the enteric valve armature and the partial sclerotisation of the folds in *D. inusitatus* can be considered as derived characters of the genus with respect to the basal state of the group (Miller 1991, Noirot 2001). The enteric valve in *N. opacus* is the only one of the described in this chapter, which has tri-radial symmetry with reduced 2 and 4 ridges, but with more than 30 pines on each fold (Figures 104-106).



References: I, II, III: proventricular ridges, c: columns, p: pulvilli, 1-4: enteric valve ridges, s: spines, cf: cuticular filaments, t: tubercles. Scale bars: 106, 109, 111 = 0.1 mm; 105, 110, 112 = 10 µm; 107, 108 = 50 µm.

Figures 105-112. Internal ornamentations of *N. opacus* and *S. trispinosus*. 105: *N. opacus* worker enteric valve (detail), 106: *N. opacus* soldier enteric valve, 107: *N. opacus* spines of soldate third proctodeal segment, 108: *N. opacus* spines of alate third proctodeal segment, 109: S. *trispinosus* worker gizzard, 110: S. *trispinosus* worker gizzard (detail), 111: S. *trispinosus* soldier gizzard, 112: S. *trispinosus* soldier gizzard (deatil).

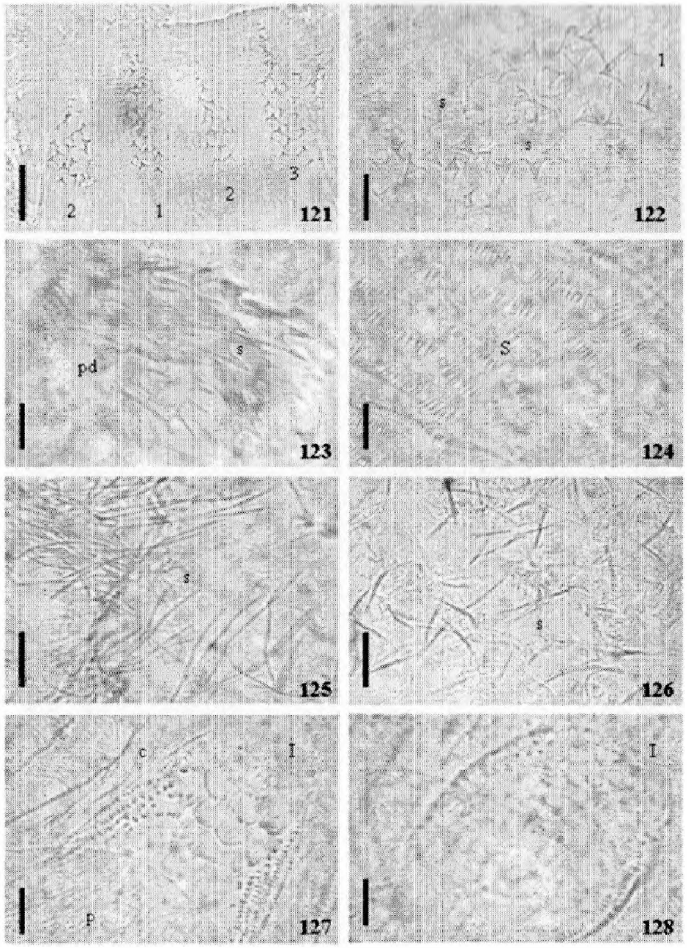
This type of symmetry is also recognized in other Termitinae genera such as *Ephelotermes* and *Genuotermes* (Miller 1991, Sands 1998, Noirot 2001, Bourguignon et al. 2008, Rocha 2013).



References: I, II, III: proventricular ridges, c: columns, p: pulvilli, 1-4: enteric valve ridges, s: spines, S: scales. Scale bars: 115, 117 = 0.1 mm; 114, 116, 118 = 10 µm; 113, 119, 120 = 50 µm.

Figures 113-120. Internal ornamentations of *S. trispinosus* and *T. bolivianus*. 113: *S. trispinosus* worker enteric valve, 114: *S. trispinosus* worker enteric valve (detail), 115: *S. trispinosus* soldier enteric valve, 116: *S. trispinosus* soldier enteric valve (detail), 117: *T. bolivianus* soldier, 118: *T. bolivianus* soldier gizzard (detail), 119: *T. bolivianus* alate gizzard, 120: *T. bolivianus* alate gizzard (detail).

The enteric valve armature located predominantly in the lumen of this segment is recognized in most Termitidae genera, but in some Apicotermitinae the more or less elaborate ornamentation is everted into the third proctodeal segment (Godoy and Torales 1999 b, Donovan et al. 2000, Donovan 2002, Bourguignon et al. 2010).



References: I, II, III: proventricular ridges, c: columns, p: pulvilli, 1-4: enteric valve ridges, s: spines, pd: pads, S: scales. Scale bars: 122-124, 127, 128 = 10 μm; 121, 125, 126 = 50 μm.

Figures 121-128. Internal ornamentations of *T. bolivianus* and *T. nigritus*. 121-122: *T. bolivianus* worker enteric valve, 123: *T. bolivianus* worker rectal valve, 124: *T. bolivianus* worker crop, 125: *T. bolivianus* cuticular filaments of alate third proctodeal segment,126: *T. bolivianus* cuticular filaments of alate fourth proctodeal segment,127: *T. nigritus* worker gizzard, 128: *T. nigritus* soldier gizzard.

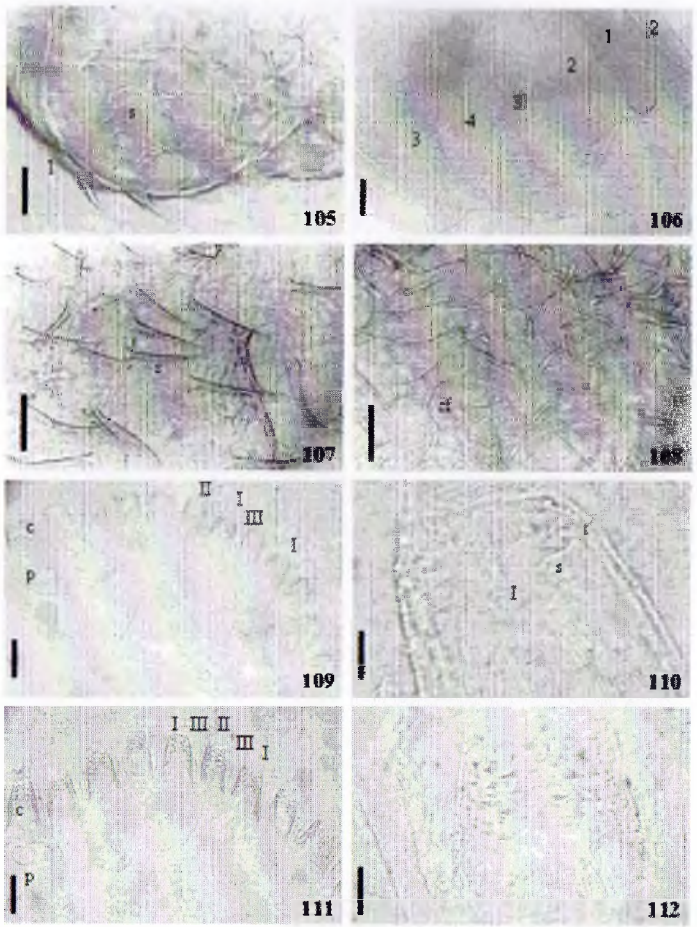
The structure observed in the species described here is considered as the probable basal state of the family (Noirot 2001). The longest spines that compose the internal armature of the enteric valve were present at the distal third of the ridges in eight species, but only in the middle third of the six *A.*

amifer and *M. strunckii* ridges and in the 1 and 3 ridges in *D. inusitatus*. Only in the three *Termes* (Figures 121, 129, 133, 134), the longest spines were distributed over the entire surface of the ridges and the number of spines of the 1 and 3 ridges was lower.

These longest spines were tapered or straight in the observed species except *N. opacus* and *S. trispinosus* where they were curved (Figures 105, 114). The distal spines directed backwards are most frequently observed in species with chitinous ornamentations and probably have the functions of preventing antiperistaltic movements and participate in the separation of the ingested material (Sands 1998, Donovan 2002). In *S. trispinosus*, the enteric valve had the lowest number of spines between the Termitinae included in this chapter. This armature was similar to the ones of other *Spinitermes* species and differs from *Divinotermes* that shows a rough surface (Mathews 1977, Godoy and Torales 1993, Carrijo 2009, Carrijo and Cancello 2011).

The subsidiary armature of the enteric valve varied from reticulated (*D. inusitatus* and *S. trispinosus*), with backwards directed scales (*A. amifer* and *M. strunckii*) and with small spines on scales (*N. opacus* and the three *Termes*). While the more developed ornamentations, everted into the third proctodeal segment in some Apicotermitinae seem to be an adaptation to true, a clear relationship of these remarkable structures with this feeding group is not clearly recognized, as in some neotropical genera of the *Anoplotermes* group with the same diet, the armature is poorly developed (*Aparatermes*) or absent (*Anoplotermes*) (Sands 1972, Fontes 1986, Donovan 2002, Bourguignon et al. 2010, 2013; Bignell 2011, Godoy *unpublished obs.*). In Termitinae with the same diet, the ornamentations are much faint and arranged in the lumen of the second proctodeal segment (Donovan 2002). Moreover, between soil-feeding neotropical Nasutitermitinae, three enteric valve ornamentation types are recognized differing in the size of the spines (Fontes 1987 a).

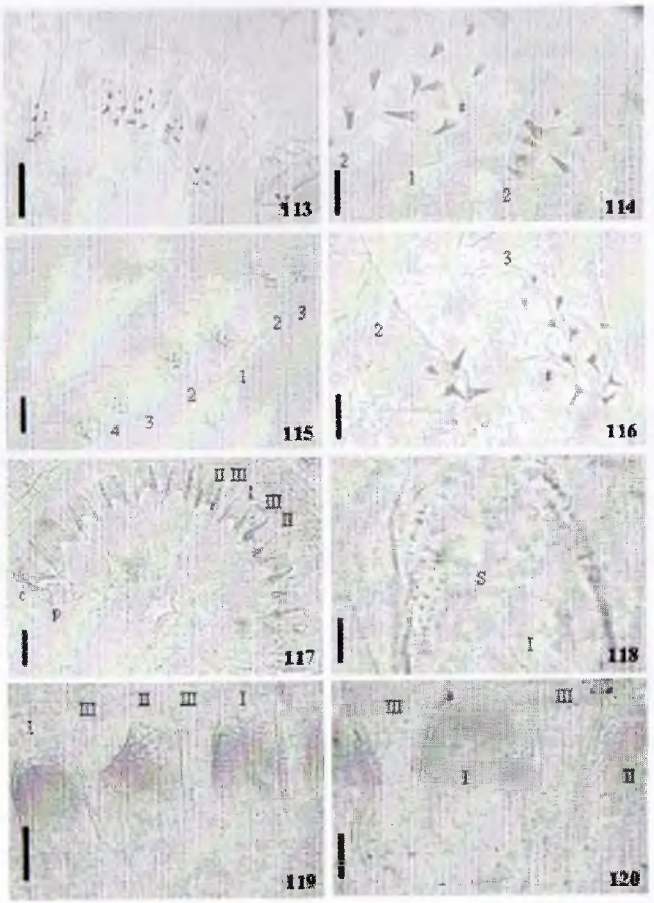
The enteric valve ornamentation was more developed in workers than in soldiers and alates, except in one species. Only in *N. opacus*, the enteric valve showed longer ridges and more numerous spines in soldiers. The number of larger spines on the enteric valve ridges showed significant differences between castes in four species, with the workers having more spines in *D. inusitatus* and *M. strunckii*, the alates in *T. bolivianus* and the soldiers in *T. riograndesis*, respectively. The existence of a membranous wall devoid of spines or scales on the enteric valve ridges and beyond them, as it was observed in the studied group, is recognized in most Termitidae genera.



References: I, II, III: proventricular ridges, c: columns, p: pulvilli, 1-4: enteric valve ridges, s: spines, cf: cuticular filaments, t: tubercles. Scale bars: 106, 109, 111 = 0.1 mm; 105, 110, 112 = 10 µm; 107, 108 = 50 µm.

Figures 105-112. Internal ornamentations of *N. opacus* and *S. trispinosus*. 105: *N. opacus* worker enteric valve (detail), 106: *N. opacus* soldier enteric valve, 107: *N. opacus* spines of soldate third proctodeal segment, 108: *N. opacus* spines of alate third proctodeal segment, 109: S. *trispinosus* worker gizzard, 110: S. *trispinosus* worker gizzard (detail), 111: S. *trispinosus* soldier gizzard, 112: S. *trispinosus* soldier gizzard (deatil).

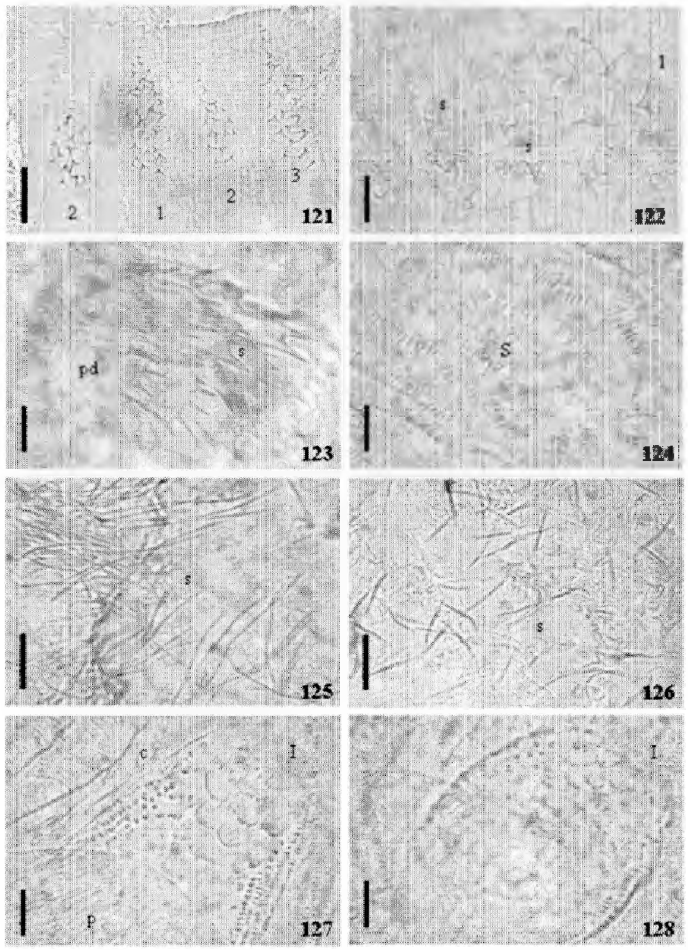
This type of symmetry is also recognized in other Termitinae genera such as *Ephelotermes* and *Genuotermes* (Miller 1991, Sands 1998, Noirot 2001, Bourguignon et al. 2008, Rocha 2013).



References: I, II, III: proventricular ridges, c: columns, p: pulvilli, 1-4: enteric valve ridges, s: spines, S: scales. Scale bars: 115, 117 = 0.1 mm; 114, 116, 118 = 10 µm; 113, 119, 120 = 50 µm.

Figures 113-120. Internal ornamentations of *S. trispinosus* and *T. bolivianus*. 113: *S. trispinosus* worker enteric valve, 114: *S. trispinosus* worker enteric valve (detail), 115: *S. trispinosus* soldier enteric valve, 116: *S. trispinosus* soldier enteric valve (detail), 117: *T. bolivianus* soldier, 118: *T. bolivianus* soldier gizzard (detail), 119: *T. bolivianus* alate gizzard, 120: *T. bolivianus* alate gizzard (detail).

The enteric valve armature located predominantly in the lumen of this segment is recognized in most Termitidae genera, but in some Apicotermitinae the more or less elaborate ornamentation is everted into the third proctodeal segment (Godoy and Torales 1999 b, Donovan et al. 2000, Donovan 2002, Bourguignon et al. 2010).



References: I, II, III: proventricular ridges, c: columns, p: pulvilli, 1-4: enteric valve ridges, s: spines, pd: pads, S: scales. Scale bars: 122-124, 127, 128 = 10 µm; 121, 125, 126 = 50 µm.

Figures 121-128. Internal ornamentations of *T. bolivianus* and *T. nigritus*. 121-122: *T. bolivianus* worker enteric valve, 123: *T. bolivianus* worker rectal valve, 124: *T. bolivianus* worker crop, 125: *T. bolivianus* cuticular filaments of alate third proctodeal segment,126: *T. bolivianus* cuticular filaments of alate fourth proctodeal segment,127: *T. nigritus* worker gizzard, 128: *T. nigritus* soldier gizzard.

The structure observed in the species described here is considered as the probable basal state of the family (Noirot 2001). The longest spines that compose the internal armature of the enteric valve were present at the distal third of the ridges in eight species, but only in the middle third of the six *A.*

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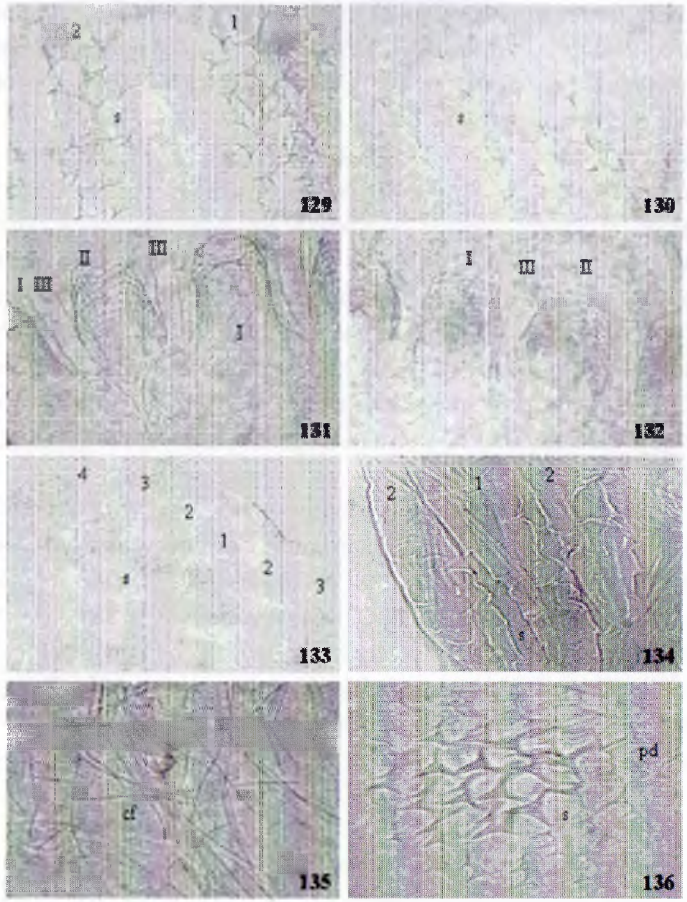
These longest spines were tapered or straight in the observed species except *N. opacus* and *S. trispinosus* where they were curved (Figures 105, 114). The distal spines directed backwards are most frequently observed in species with chitinous ornamentations and probably have the functions of preventing antiperistaltic movements and participate in the separation of the ingested material (Sands 1998, Donovan 2002). In *S. trispinosus*, the enteric valve had the lowest number of spines between the Termitinae included in this chapter. This armature was similar to the ones of other *Spinitermes* species and differs from *Divinotermes* that shows a rough surface (Mathews 1977, Godoy and Torales 1993, Carrijo 2009, Carrijo and Cancello 2011).

The subsidiary armature of the enteric valve varied from reticulated (*D. inusitatus* and *S. trispinosus*), with backwards directed scales (*A. amifer* and *M. strunckii*) and with small spines on scales (*N. opacus* and the three *Termes*). While the more developed ornamentations, everted into the third proctodeal segment in some Apicotermitinae seem to be an adaptation to true, a clear relationship of these remarkable structures with this feeding group is not clearly recognized, as in some neotropical genera of the *Anoplotermes* group with the same diet, the armature is poorly developed (*Aparatermes*) or absent (*Anoplotermes*) (Sands 1972, Fontes 1986, Donovan 2002, Bourguignon et al. 2010, 2013; Bignell 2011, Godoy *unpublished obs.*). In Termitinae with the same diet, the ornamentations are much faint and arranged in the lumen of the second proctodeal segment (Donovan 2002). Moreover, between soil-feeding neotropical Nasutitermitinae, three enteric valve ornamentation types are recognized differing in the size of the spines (Fontes 1987 a).

The enteric valve ornamentation was more developed in workers than in soldiers and alates, except in one species. Only in *N. opacus*, the enteric valve showed longer ridges and more numerous spines in soldiers. The number of larger spines on the enteric valve ridges showed significant differences between castes in four species, with the workers having more spines in *D. inusitatus* and *M. strunckii*, the alates in *T. bolivianus* and the soldiers in *T. riograndesis*, respectively. The existence of a membranous wall devoid of spines or scales on the enteric valve ridges and beyond them, as it was observed in the studied group, is recognized in most Termitidae genera.



References: I, II, III: proventricular ridges, c: columns, p: pulvilli, 1-4: enteric valve ridges, s: spines, pd: pads, S: scales, cf: cuticular filaments. Scale bars: 12-124, 127, 128 = 10 µm; 121, 125, 126 = 50 µm.

Figures 129-136. Internal ornamentations of *T. nigritus* and *T. riograndensis*. 129: *T. nigritus* worker enteric valve, 130: *T. nigritus* soldier enteric valve, 131: *T. riograndensis* worker gizzard, 132: *T. riograndensis* alate gizzard, 133: *T. riograndensis* soldier enteric valve, 134: *T. riograndensis* alate enteric valve, 135: *T. riograndensis* cuticular filaments of alate third proctodeal segment, 136: *T. riograndensis* alate rectal valve.

However, in the *Cubitermes* group, in addition to the six major ridges, other six subsidiary ridges armed with spines are recognized. This feature is regarded as one of the two autoapomorphies of the group (Sands 1998, Noirot 2001, Bitsch and Noirot 2002).

The third proctodeal segment showed an internal ornamentation composed of long, thin cuticular spines in alates of the five species in which this caste was examined (Figures 88, 108, 125). These structures are also found in *M. strunckii* and *N. opacus* workers and soldiers (Figure 107), but absent in neuters of the other species. The ornamentation was weaker in *N. opacus* alates than in the other castes. Similar spines have been recorded in other *Neocapritermes* species and other Termitinae genera as *Amitermes*, *Cylindrotermes* and *Drepanotermes*. These chitinous spines making protrusion in the intestinal lumen act as attachment sites for intestinal symbionts and are probably also involved in the separation of the intestinal contents facilitating the retention of the finest materials in the periphery of the third proctodeal segment and the faster advance of the coarse particles toward the colon (Bignell 2000, 2011; Noirot 2001, Donovan 2002). They are also important in taxonomy and phylogeny in some Termitinae groups as *Pericapritermes* where complex chitinous structures are observed (Noirot 2001).

The presence of long cuticular spines in the proximal colon of *A. amifer* workers and soldiers (Figure 78) had not been registered previously for the *Amitermes* genus. Also in *T. bolivianus* and *T. riograndensis* alates, thin cuticular spines were recognized in the anterior portion of the fourth proctodeal segment (Figure 126).

The rectal valve was armed with six ridges carrying scales and spines in the investigated species of the *Termes* group (Figures 99, 123, 136). The presence of an armature is considered as a plesiomorphic character in termites that suffered regressions in several groups (Noirot 2001). By contrast, the ornamentation was reduced in species of the *Amitermes* group analyzed in this chapter (Figures 79, 80). Similarly to these observations, Noirot (2001) indicates that the rectal valve is always armed in the *Termes* group, while for *Amitermes* group this structure is usually reduced or absent. However, Bitsch and Noirot (2002) indicate the presence of unarmed rectal valves in the genera *Termes* and *Microcerotermes* and armed ones in the genus *Amitermes*, without mention the analyzed species.

Conclusion

The interpretation of the intestinal patterns and morphological changes in different termite groups of in relation to diet is difficult because of the remarkable diversity observed within each feeding group, family and subfamily.

However, the data presented in this chapter have shown the effects of social polymorphism in the intestinal structure of the individuals and allowed to recognize differences between castes of each species in the analyzed Termitinae. Since alates are considered the most anatomically generalized individuals of the colonies, the characters observed in workers and soldiers could be regarded as modifications related to the alimentary items consumed or the feeding mode of each caste. However, these relationships explain only to some extent the observed intestinal morphological variations. In termites, the noteworthy gut diversity, superior than that observed in the external morphology of the group is undoubtedly related to other aspects that certainly influence the intestinal design as the composition and location of the symbiotic communities and the complex physiological mechanisms taking place inside the alimentary canal.

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To G. Torales and J. Coronel for the identification of the samples. To J. Liotta and B. Giacosa for the gift of *O. brevicorniger* specimens. Supported by Secretaria General de Ciencia y Técnica, Universidad Nacional del Nordeste, Corrientes, Argentina.

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