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Studies on *Reticulitermes lucifugus* Rossi (Isoptera: Rhinotermitidae): a review of associated hindgut flagellates and investigations on protist species of the Sicilian subspecies, *R. lucifugus* "Sicily"

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Abstract
The present study was conducted on protist species from *R. lucifugus* "Sicily" subspecies in a field site of Palermo (Sicily, Italy) on June 2015. Since protist species associated with *R. lucifugus* in Europe are still confused and recent revisions are lacking, this work aims at achieving greater clarity on these symbiosis trough a review of the literature in light of new phylogenetic studies on this termite species. The purpose of this work was also to highlight and quantify protists associated with the Sicilian subspecies, *R. l.* "Sicily". Results pointed out the real protist community attributable to *R. lucifugus* from the literature and showed 13 protist species detected in the subspecies *R. l.* "Sicily". These species were consistent with previous reports except for *Spironympha* sp., mentioned for the first time from this termite. Total protist population estimates was 26,814.71 ± 1,121.92 protists per termite. *Dinenympha exilis* and *D. gracilis* were found in greater abundance with relative species abundance of 17%. *Monocercomonas termitis*, *Microjoenia hexamitoides*, *Trichonympha minor* and *Pyrsonympha flagellata* could be used to distinguish *R. lucifugus* because these protists are not found in other *Reticulitermes* species.

Keywords: Intestinal protozoa, subterranean termites, protist abundance

1. Introduction
Reticulitermes lucifugus Rossi (Blattodea, Termitoidae, Rhinotermitidae), initially named *Termes lucifugus* was the first *Reticulitermes* species described in Europe, from samples collected in Tuscany, Italy [1-3]. In the past, this species was believed to be the only one of the genus *Reticulitermes* living in Europe. However, a taxonomic and phylogenetic re-analysis of the genus *Reticulitermes* trough morphological, chemical, and molecular studies has led to identification of different *Reticulitermes* species where in the past they were reported as *R. lucifugus*, showing a different scenario concerning the distribution area of this species [3]. In particular, a total of seven *Reticulitermes* species were identified in many localities: *R. lucifugus* Rossi in Italy and southeastern France, *R. grassei* (Clément) in southwestern France (Provence), northwestern and southern Spain and Portugal, *R. banyulensis* (Clément) in southern France (Roussillon) and northeastern Spain, *R. balkanensis* Clément in the Balkans, *R. santonensis* Feytaud (= *R. flavipes*) in western France, *R. urbis* Bagnères & Clément in Italy, southeastern France and western Greece, Peloponnese and Croatia, and *R. flavipes* Kollar (introduced to Europe) in Germany and Italy [3-9].
In Italy, *R. lucifugus* is currently the most abundant species found along the western coast and in Sicily [10-12, 7]. This species has two subspecies known as *R. lucifugus lucifugus* on the peninsula and *R. lucifugus corsicus* in the Sardo-Corsican area and Tuscanian coasts [13, 6, 14; 8, 9]. In addition, a third taxon of subspecific level, named *R. lucifugus* "Sicily" has been found in some areas of Sicily divergent from the other Italian subspecies for genetic and morphometric aspects [15, 14, 16].
Reticulitermes species can be distinguished also on the basis of their intestinal flagellates, that are specific to each termite species [17-24]. In addition, protist species common to some *Reticulitermes* species differ in their proportion in the community composition of each termite species [22, 24]. The genus *Reticulitermes* has a variety of symbiotic protozoa living in the hindgut. More than 400 species of flagellates have been reported [20, 25].

These protozoa comprise flagellates belonging to the phylum Parabasalia, orders Tritrichomononadida ordo nov., Trichomonadida Kirby, Honigbergiellida ordo nov., Spirotrichonymphida Grassé, Cristamonadida Brugerolle & Patterson and Trichonymphyda Poche, and phylum Preaxostyla, order Oxymonadida Grassé [26, 27]. The first cytological description of protist species was made in *R. lucifugus*, and flagellates and their identification on this species in Europe have been documented in literature [28-36, 20]. However, we think that a revision of the protist communities living in *R. lucifugus* based on the recent phylogenetic studies of the genus *Reticulitermes* in Europe would be necessary to avoid confusion on the flagellates attributed to this species and for a better determination of termite species.

This work aims at achieving greater clarity on flagellate communities of *R. lucifugus* providing a review of the existing literature to which many researchers are still referring, emphasizing the actual protist species of this termite on the basis of its real distribution area. Furthermore, the objective of the present study was to point out, by means of a qualitative and quantitative study, the protist species detected in *R. lucifugus* "Sicily", where we have previously studied the temporal variation of some of its hindgut flagellate species and total population [37]. The study has been carried out on the worker caste, that it is well known having all representatives of the protist community [38, 24].

2. Materials and methods

To provide an overview of protist communities reported from *R. lucifugus* we used the existing literature concerning this topic. To perform the experimental study we used the methodology reported in Lo Pinto *et al*. [37].

2.1 Insects

Termites of the species *R. lucifugus* were collected from a field site of Palermo (Sicily, Italy) in June 2015. Blocks of ten fir tablets (each 10 cm x 10cm x 1 cm), held together by an elastic band and installed inside open-bottomed buckets placed in the ground at 30 cm depth, approximately one meter from each other, were used. The blocks were covered with crocks to maintain the necessary moisture. Among termites collected from blocks brought to the laboratory (25°C, 60% r.h) workers with dark brown and distended abdomens (not recently molted) were chosen to examine their hindgut contents. Hindguts were removed by gently holding the termite by the thorax and removing the last two abdominal segments by using fine-tipped forceps. The gut contents from 3 workers were pooled to form a sample and 34 replicates were made.

2.2 Protists

To count protists we used the technique described in Mannesmann [38]: termite hindgut contents were put into 60 µl salt solution (NaCl 0.8 g, KCl 0.02 g, $CaCl_2$ 0.02 g, $NaHCO_3$ 0.01 g in 100 ml distilled water) in which neutral red had been dissolved (0.5 ml of 1% aqueous neutral red solution into 10 ml salt solution). A small amount of the samples was placed in a cell counting chamber (such as Bürker's chamber HBG Germany). Immediately after filling the counting chamber, two counting sections of the chamber were chosen at random and counting of the protists (one measurement per counting section) was performed under a phase contrast microscope at 400x. An Axiophot microscope at 400x magnification equipped with an AxioCam digital camera MR5 (Zeiss) and a Laser scanning microscope LSM510 (Zeiss) were used to acquire images of protists. The protist species were reconized using the original and revised species descriptions [39, 29, 40-43]. The calculation of the number of protists of each identified species per hindgut was based on the weight (volume) of the solution, the three hindguts, the volume of the counted area of the chamber and the mean of the two counts.

The calculation was made by estimating the average number of protists per hindgut (X_F) as follows: $X_F = G*n/V*3$, where G is volume of solution containing the three hindguts (60 µl); n is mean of the two counts; V is volume of the counted area (in µl).

Species abundances (average number of each protist species per termite) and proportions (species abundances relative to the total number of the hindgut protists) were calculated and compared, in order to evaluate whether species, even if quantitatively different, had the same significance in terms of presence in the hindgut of the host.

2.3 Statistical analyses

To compare data for protist species abundances and proportions we used nonparametric procedures because normality conditions (checked with Kolmogorov-Smirnov tests) were not always met. Thus the data were analyzed using Kruskal-Wallis one way analysis of variance on ranks, and significant differences between the means were separated by a post hoc test for multiple-sample comparisons (2-tailed) (α = 0.05). Species abundance data were square root transformed (x+0.5) and proportional data were arcsine transformed before data analysis. However, in figures and tables untransformed data are provided for interpretation. All statistical analyses were performed using Statistica 7.0 for Windows Package [44].

3. Results

3.1 Protist species from *R. lucifugus:* a review of the literature

The overview of protist species attributed to *R. lucifugus*, historically considered the only *Reticulitermes* species in Europe, showed 31 protist species reported from different European localities (Table 1).

The taxonomic and phylogenetic re-analysis of the genus *Reticulitermes* highlighted that *R. lucifugus* is distributed in Italy and southeastern France and not in other localities of Europe where this species was reported because different *Reticulitermes* species were believed wrongly this species. Consequently, the protist species attributable to *R. lucifugus* were those reported from its actual area of distribution for a total of 16 species, missing those from Yugoslavia, Romania, Greece, southwestern France and Portugal (Table 2).

Table 1: Protist species attributed to *Reticulitermes lucifugus* Rossi from European localities reported in the literature and in this study (*R. lucifugus* "Sicily").

Protist species	This study	Previous literature
Pyrsonymphidae		
Dinenympha aculeata Georgévitch	-	Yugoslavia (Dalmatia) [45]
D. aviformis Georgévitch	-	Yugoslavia (Dalmatia) [45]
D. exilis Koidzumi	+	Italy (Genoa[1]), Portugal (Freixial) [36] and Yugoslavia (Dalmatia) [32]
D. fimbriata Kirby	+	France (Corse)?, Italy (Fiascherino, Genoa[1] and Istria[2]) [36] and Yugoslavia (Dalmatia) [32]
D. gracilis Leidy	+	France (Corse), Italy (Catania, Fiascherino, Francavilla Genoa[1], Istria[2] and Rome), Portugal (Freixial), [28, 34, 36] and Yugoslavia (Serbia and Dalmatia) [33, 32]
D. sp.	-	Yugoslavia (Serbia) [33]
Pyrsonympha elongata Georgévitch	-	Yugoslavia (Serbia and Dalmatia) [33, 45]
P. elongata var. *oblensis* Georgévitch	-	Yugoslavia (Serbia and Dalmatia) [33, 45]
P. flagellata Grassi	+	France (Corse and Paris), Italy (Catania, Fiascherino, Francavilla, Genoa[1], Istria[2] and Rome) and Portugal (Freixial) [28, 34]; Hollande [46, 36]
P. granulata Powell	-	Greece (Janina) [32] and Yugoslavia (Serbia and Dalmatia) [33, 45]
P. major Powell	+	Greece (Janina) [32]
P. modesta Koidzumi	-	Yugoslavia (Serbia) [33]
P. modesta var. *rostrata* Georgévitch	-	Yugoslavia (Dalmatia) [45]
P. minor Powell	+	Greece (Janina) [32]
P. sp.	-	France (Corse), Italy (Fiascherino, Francavilla Genoa[1] and Istria[2]) and Portugal (Freixial) [36]
Monocercomonadidae		
Monocercomonas termitis (Grassi)	+	Italy (Catania) [28]
Trichomonadidae		
Trichomonas trypanoides Duboscq & Grassé	-	France (Bordeaux) [31]
Hexamastigidae		
Hexamastix termitis (Grassi)	-	France (Corse), Italy (Fiascherino, Genoa[1] and Istria[2]) and Portugal (Freixial) [36]
Holomastigotoididae		
Microjoenia hexamitoides Grassi	+	Italy (Catania) [28]
M. sp.	-	Italy (Fiascherino, Genoa[1] and Istria[2]) and Portugal (Freixial) [36]
Holomastigotes elongatum Grassi	+	Italy (Catania, Fiascherino, Francavilla) [28, 36], Romania (Bucharest) [35] and Yugoslavia (Serbia and Dalmatia) [33, 32]
Spirotrichonympha crinita Ionescu & Murgoci	-	Romania (Bucharest) [47]
S. flagellata (Grassi)	+	France (Corse), Italy (Catania, Fiascherino, Francavilla, Genoa[1], Istria[2] and Rome), Portugal (Freixial), [29, 34, 36], Greece (Janina) [32], Romania (Bucharest) [47] and Yugoslavia (Serbia and Dalmatia) [33, 45]
S. metchnikovi (Franca)	-	Portugal (Colares) [30]
S. segmentata Georgévitch	-	Yugoslavia (Serbia) [33]
Spironympha kofoidi Koidzumi	+	-
Trichonymphidae		
Trichonympha agilis Leidy	+	France (Corse), Italy (Catania, Fiascherino, Francavilla, Genoa[1] and Rome), Portugal (Freixial and Colares) [28, 30, 29, 48, 34, 36], Romania (Bucharest) [35] and Yugoslavia (Dalmatia) [32]
T. agilis var. *danubica* Ionescu & Murgoci	-	Romania (Bucharest) [35]
T. liviae Ghidini	-	Italy (Rome) [34]
T. minor Grassi & Foà	+	Italy (Catania and Rome) [29, 48, 34]
T. serbica Georgévitch	-	Yugoslavia (Serbia) [33]

(1) Note: termites were collected from a building in Munich (Germany) but they have been introduced from Genoa (Italy) [36]

(2) Note: Italian Istria [36]

Table 2: Protist species reported for true *Reticulitermes lucifugus* Rossi colonies from Italian and Corsican localities (current distribution area of this species).

Protist species	Italian regions							France
	Sicily		Abruzzo	Liguria		Friuli	Latium	Corse
	*PAL	CAT	FRA	FIA	GEO	IST	ROM	FAV
D. exilis	+	-	-	-	+	-	-	-
D. fimbriata	+	-	-	+	+	+	-	+?
D. gracilis	+	+	+	+	+	+	+	+
P. flagellata	+	+	+	+	+	+	+	+
P. minor	+	-	-	-	-	-	-	-
P. major	+	-	-	-	-	-	-	-
P. sp.	-	-	+	+	+	+	-	+
M. termitis	+	+	-	-	-	-	-	-
H. termitis	-	-	-	+	+	+	-	+
M. hexamitoides	+	+	-	-	-	-	-	-
H. elongatum	+	+	+	+	-	-	-	-
S. flagellata	+	+	+	+	+	+	+	+
S. kofoidi	+	-	-	-	-	-	-	-
T. agilis	+	+	+	+	+	-	+	+
T. liviae	-	-	-	-	-	-	+	-
T. minor	+	+	-	-	-	-	+	-
No. of species consistent with our results		8	5	6	6	4	4	5

*PAL=Palermo (this study) - CAT=Catania [28] - FIA=Fiascherino, FRA=Francavilla, GEO=Genoa, IST=Istria, ROM=Rome and FAV=Favone [36]

3.2 Protist species of *R. lucifugus* "Sicily": a qualitative and quantitative study

The present study found 13 protist species in *R. lucifugus* "Sicily" as *Dinenympha exilis* Koidzumi, *D. fimbriata* Kirby, *D. gracilis* Leidy, *Pyrsonympha flagellata* Grassi, *P. minor* Powell, *P. major* Powell, *Monocercomonas termitis* (Grassi), *Microjoenia hexamitoides* Grassi, *Holomastigotes elongatum* Grassi, *Spirothriconympha flagellate* (Grassi), *Spironympha kofoidi* Koidzumi, *Trichonympha agilis* Leidy, and *T. minor* Grassi & Foà. They were consistent with previous reports [28, 36], except for *S. kofoidi*, mentioned for the first time in this termite species. We noted a close similarity between protist species found in our study and those reported for *R. lucifugus* from Italian and Corsican localities, except for *P. minor*, *P. major* and *S. kofoidi* which are not mentioned for these sites. Specifically, the highest congruence is found between our collection (Palermo)and Catania (where 8 species matched), followed by Fiascherino and Genoa (6 species), Francavilla and Favone (Corse) (5 species), and Istria and Rome (4 species) (Table 2).

The total protist population was 26,814.71 ± 1,121.92 protists per termite (n=34) (mean ± SE). The mean protist species abundances are summarized in Table 3. *D. exilis* was the most abundant flagellate in the hindgut termite, followed by *D. gracilis*, *M. termitis*, *P. minor*, *M. hexamitoides*, *D. fimbriata*, *P. flagellata*, *H. elongatum*, *P. major*, *S. kofoidi, T. agilis*, *S. flagellata* and *T. minor*. Significant differences among the means of protist abundances were found (Kruskal-Wallis test: H = 270.11, df = 12, N = 442, P = 0.000). The post hoc test for multiple-sample comparisons shows that protist species can be divided into two groups statistically different, where within each group they did not differ significantly. The groups are: group 1, consisting of *D. fimbriata*, *D. gracilis, D. exilis*, *M. termitis*, *M. hexamitoides* and *P. minor,* that was more abundant and group 2, consisting of *S. flagellata*, *S. kofoidi*, *T. agilis*, *T. minor*, *P. flagellata*, *H. elongatum* and *P. major,* that was minor abundant (Table 3).

Table 3: Species abundances (mean ± SE) of flagellates per hindgut of *Reticulitermes lucifugus* Rossi workers from Sicily (Italy).

Protist species	Mean ± SE, n=34
Dinenympha fimbriata	2686.76 ± 342.24 ^{ab}
D. gracilis	4539.70 ± 299.42 ^{a}
D. exilis	4619.11 ± 426.23 ^{a}
Monocercomonas termitis	3666.17 ± 319.12 ^{a}
Microjoenia hexamitoides	3176.47 ± 414.92 ^{a}
Holomastigotes elongatum	1085.29 ± 155.65 ^{bc}
Spirotrichonympha flagellata	463.23 ± 77.14 ^{c}
Spironympha kofoidi	661.76 ± 115.93 ^{c}
Trichonympha agilis	502.94 ± 127.12 ^{c}
T. minor	317.65 ± 113.25 ^{c}
Pyrsonympha flagellata	1177.94 ± 152.01 ^{bc}
P. minor	3216.17 ± 311.10 ^{a}
P. major	701.47 ± 124.86 ^{c}

Means (±SE) followed by the same letters do not differ significantly from each other at 0.05 of significance level (Kruskal-Wallis test followed post hoc test for multiple-sample comparisons).

Relative species abundances of protists occurring in the hindgut community of *R. lucifugus* "Sicily" showed a highest percentage of *D. exilis* followed by *D. gracilis*, *M. termitis*, *P. minor*, *M. hexamitoides*, *D. fimbriata*, *P. flagellata*, *H. elongatum*, *P. major*, *S. kofoidi*, *T. agilis*, *S. flagellata* and *T. minor.* Significant differences among the percentage means of the flagellate species were detected (Kruskal-Wallis test: H = 276.61, P = 0.000). The post hoc test for multiple-sample comparisons showed significant differences between the same groups mentioned for the species abundances (Fig. 1).

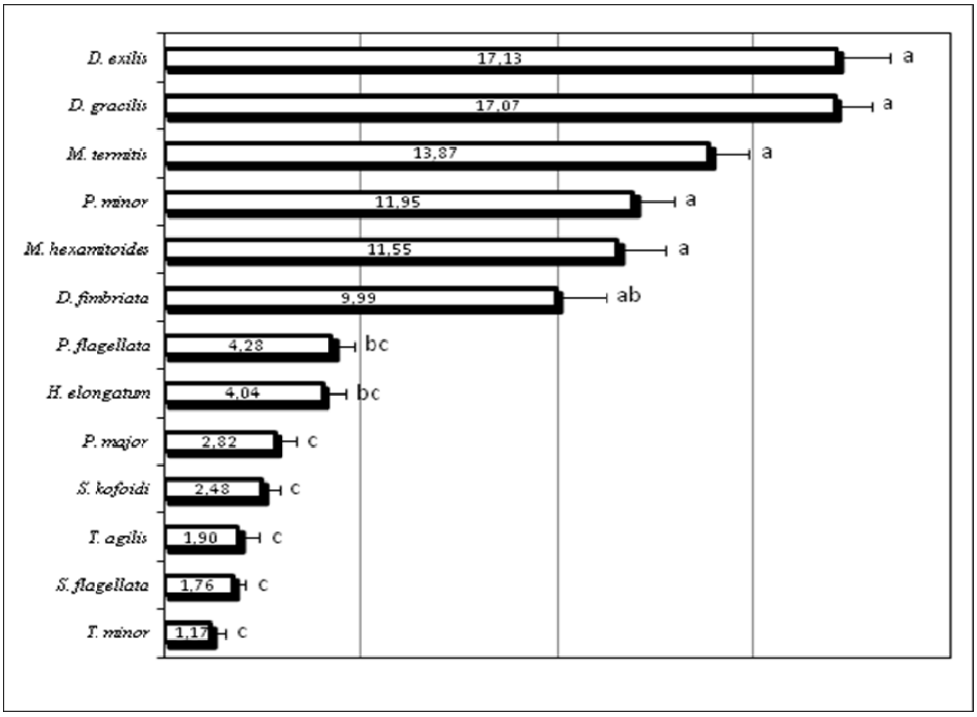

Fig 1: Relative species abundances (mean ± SE percentage) of protists occurring in the hindgut community of *Reticulitermes lucifugus* Rossi "Sicily". Bars sharing the same letters were not significantly different at 0.05 of significance level, n=34 of each species (Kruskal-Wallis test followed post hoc test for multiple-sample comparisons).

4. Discussion

Review of previous studies on the *R. lucifugus* flagellates from different European localities listed 31 protist species. Yamin [20] in a similar study reported 24 protist species from *R. lucifugus* and many studies on the termite symbiosis refer to it. However the reallocation in Europe of *Reticulitermes* taxa, supported by phylogenetic studies, evidences a different pattern: *R. lucifugus* is distributed only in Italy and southeastern France. The recent classification of taxonomic groups is of fundamental importance to assign exactly the protist communities to the different *Reticulitermes* species. In this paper we pointed out that the protist communities attributable to *R. lucifugus* are those reported for Italian and southeastern French areas only.

In the present study on *R. lucifugus* "Sicily" we found protist species previously reported from *R. lucifugus* [28, 29, 48, 34, 36, 20] except for *S. kofoidi* that was not mentioned in this termite species. Also, many of these species are reported in literature for *R. lucifugus* from Italian and Corsican localities, that are the actual distribution areas of this termite. Some differences between our results (Palermo site) and those of the sites of the present-day distribution of *R. lucifugus* might depend by geographical locations of colonies that influence the presence or lacking of some protist species in the gut flagellate communities of termites [36, 49]. As could have been expected, most of protist species found in this work were described in *R. lucifugus* from Catania (Sicily) [28, 29, 48]. Specifically, in comparison with Catania, our results confirmed the presence of *D. gracilis*, *P. flagellata*, *M. termitis*, *M. hexamitoides*, *H. elongatum*, *S. flagellata*, *T. agilis* and *T. minor.* We also found *D. exilis*, *D. fimbriata*, *P. minor*, *P. major* and *S. kofoidi* not reported in Catania site. The largest number of species detected in our study than Catania (detected in the past century), could be an effect of biological and environmental changes that influenced the gut microbial community of *R. lucifugus*. In addition, our work highlighted that *M. termitis* and *M. hexamitoides* were found only in Sicily, thus we can assume that these protist species are specific for the Sicilian *R. lucifugus*. *T. minor* which is specific for *R. lucifugus* [34] was found only in Sicily and Rome. Also we found *P. minor* and *P. major* reported in Greece [32] but not in other Italian and Corsican localities, and *S. kofoidi*, that was not reported for *R. lucifugus*.

Our protist population estimates were lower than those previously reported from *R. lucifugus* colonies from different localities (Fiascherino, Francavilla, Genoa, Istria, Favone) which ranged from 145,281 to 59,106 at 26 °C and from 175,110 to 57,954 at 28 °C [36]. Comparing protist species abundances with those reported by Mannesmann [36] (*P. flagellata*, *D. gracilis, D. fimbriata*, *S. flagellata* and *T. agilis*) our estimates were lower than those of all localities at 26 °C and 28 °C, except for *D. fimbriata* which was higher with the exception for Fiascherino locality only. These different results are probably due to the environmental diversity of the collection sites [49, 22].

In our study we found significant differences on the relative abundance between two groups of protist species (group 1: *D. fimbriata*, *D. gracilis, D. exilis*, *M. termitis*, *M. hexamitoides* and *P. minor,* and group 2: *S. flagellata*, *S. kofoidi*, *T. agilis*, *T. minor*, *P. flagellata*, *H. elongatum* and *P. major*), and not significant within the groups. It is likely that within each group, the members had the same impact in the flagellate community. This may be due to the role that each species plays in the termite digestion process (cellulolytic or not cellulolytic) [50, 25, 23, 51, 27] and/or to an adaptative mechanism of termite survival strategy to overcome the loss of some protist species under unfavourable conditions (e.g. starvation, high temperature, etc.). For example, some species may be a source of nutrition for other protist species when their hosts are exposed to inadequate nutrition [52, 49, 23]. In our results, there were no significant differences between the relative abundances of *D. fimbriata* and *P. minor* and this is consistent with previous reports of a close association between the genera to which the two species belong [23; 38; 49]. Also, our

data showed that *Dinenympha* species, *M. termitis, P. minor* and *M. hexamitoides* form the most represented group in the hindgut protist population.

On the basis of the relative abundance of the protist species in their community, some species can be used as indicators to separate *Reticulitermes* species. Lewis and Forschler [24] have chosen *D. fimbriata*, *D. gracilis*, *Pyrsonympha* spp. and *T. agilis* (defined indicator protists) in relation to their greater relative abundance in *R. flavipes*, *R. virginicus* and *R. hageni* to separate these species. Cook and Gold [22] used *D. fimbriata*, *D. gracilis*, *T. agilis* and *P. minor* to distinguish *R. flavipes* and *R. virginicus*. Comparing our results on protist species considered as indicators by the previous authors, some differences emerged. For instance, *D. fimbriata* (9.9% our data) was in *R. hageni* (Banks) 27%, in *R. flavipes* 10% (work of 2004) and 15-25% (work of 1999) and in *R. virginicus* 1.5% (work of 2004) and 5-10%(work of 1999), *D. gracilis* (17.1% our data) in *R. flavipes* 30-50% (work of 1999) and 57% (work of 2004) and it was absent in *R. virginicus* and *R. hageni*, *T. agilis* (1.90% our data) in *R. virginicus* 19% (work of 2004) and 30% (work of 1999), in *R. hageni* 6.7% and in *R. flavipes* 3.7%; in relation to *Pyrsonympha* spp., *P. minor* (11.9% our data) was in *R. virginicus* 3-15% (work of 1999) and 5.9% (work of 2004), in *R. hageni* 26.3% and absent in *R. flavipes, P. major* (2.8% our data) was in *R. flavipes* 2.5% and absent in *R. virginicus* and *R. hageni*. We exclude *P. vertens* mentioned in previous works but not detected in our observations. In addition, comparing protist species which are not considered as indicators, *H. elongatum* (4.0% our data) was both in *R. flavipes* and in *R. virginicus* (Banks) 5%, *S. flagellata* (1.8% our data) was in *R. virginicus* 7%. In addition, the relative abundance of *D. exilis* representing 17.13±1.38% of the protist population in *R. lucifugus* (the greatest abundance) could easily distinguish this termite species because this protist is present in much lower numbers than in *R. speratus* (about 50%) [53], and it is not found in *R. flavipes*, *R. virginicus* and *R. hageni*.

Since the protist community structure might be used for termite identification, future studies on Italian *Reticulitermes* species and subspecies about the identification of their hindgut protist species and relative abundance are needed for a better characterization of termite species. Nevertheless, to provide a more clear-cut picture of the *Reticulitermes* taxa, these observations should be compared with molecular genetic studies based on the analyses of mitochondrial DNA of termite species and subspecies.

5. Conclusion

This paper highlighted that protist communities attributable to *R. lucifugus* are those reported for Italian and southeastern French areas only, because In light of recent studies on the genus *Reticulitermes,* the literature on *R. lucifugus* from the rest of Europe can be referred to other *Reticulitermes* species.

The experimental study on *R. lucifugus* "Sicily" showed protist species already known for *R. lucifugus* and some protist species, as *M. termitis* and *M. hexamitoides,* found only for the Sicilian subspecies, then they are specific for this subspecies. Since *T. minor* and *P. flagellata* found in this study were not reported for other *Reticulitermes* species, together previous two species they could be considered peculiar species for *R. lucifugus*. The results highlighted also the utility of using the relative abundance of protist species as indicators to identify R. lucifugus when comparing flagellate communities from different termite species.

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7. References

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