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INTEGRATIVE TAXONOMY: A STUDY ON SOME OF VICTORIA LAND'S ANTARCTIC MITE SPECIES

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To my beloved family and friends

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ABSTRACT

The extremely inhospitable Antarctic ecosystems confine plants and invertebrates to sparse and restricted ice-free areas. These species survived for millions of years in isolated refugia where population divergence and differentiation can occur, potentially resulting in speciation. The limited dispersal abilities of invertebrate species combined with their specific habitat requirements and the substantial geographical barriers can drastically reduce the gene flow between different populations, resulting in high genetic differentiation between clusters of individuals. With more than 100 described species, mites are surely the most diverse invertebrate group of Continental Antarctica. Among them, the free-living genus *Stereotydeus* Berlese, 1901 (Acari: Prostigmata) is represented by 6 Antarctic species of which 5 occur along the coastal zones of Victoria Land and the Transantarctic Mountains. In order to examine the biodiversity and the phylogeographic distribution ranges of *Stereotydeus* spp. across Victoria Land, I conducted an integrated analysis of the genus through phylogenetic, morphological and population genetics studies on specimens collected from nine localities in Victoria Land. I sequenced the second part of the cytochrome *c* oxidase subunit *I* mitochondrial gene (*cox1*) and a fragment of the 28S ribosomal RNA-encoding gene using mite-specific primers. I morphologically identified and described two novel *Stereotydeus* species from central and south Victoria Land. While the relationships between the *cox1* haplotypes from North Victoria Land are well defined, the distribution of the central-southern species appears more complex. This suggests a possible common evolutionary history in many isolated glacial refugia, with scarce gene flow even within populations probably resulting from inter/intra-specific events influenced by several abiotic/biotic factors. Recent threats to Antarctic biodiversity like accelerated climate change, pollution, biological invasions and the increase of human activities have caused increased calls for adequate conservation measures. Establishing a new distribution map for the *Stereotydeus* species of Victoria Land may help lay the foundations for future decisions in matters of protection and conservation of the unique terrestrial fauna of Antarctica.

1. INTRODUCTION

1.1 The species concept

During the years, many different tools were developed and used by taxonomists, systematists and geneticists to define the species concept. When the determination of a species is difficult from the morphological point of view, the analysis of the molecular structure and variation can be a useful tool in the identification of the species and also discover the presence of cryptic species. Moreover, together with population genetics analyses, it is possible to highlight the connections between the populations' objects of the study (Thorp *et al.* 2015). The debate around the definition of the species concept arose during the last century and is still ongoing. Today, we have several different and all valid ways to define a species and sometimes the different species definitions worked together to describe the populations and the resultant biodiversity. The biological, phylogenetic and evolutionary species concepts are among the most widely accepted criteria for delineating species. The biological species concept proposed by Mayr (1942) is by far the most widely used concept to define a species in many fields of biology (e.g. conservation biology, wildlife management, etc. (Thorp *et al.* 2015)). According to this concept, species are defined as groups of actually or potentially interbreeding natural populations (producing viable and fertile offspring) which are reproductively isolated from other such groups (Mayr 1942; Dobzhansky 1950). This concept is being predominantly accepted for eukaryotes with a sexual reproduction system because of their ability to mating and interbreed with an exchange of genes. According to this vision, it is possible to see a species also as a collection of gene variants and so, as the largest gene pool possible under natural conditions connecting the biological species concept to evolutionary processes. However, limiting the species definition to the biological concept only can be risky because in doing so, fundamental elements which play a role in defining species are not taken into consideration. In the end, it is important to establish a criterion or set of criteria in order to study the speciation process itself. An important limitation to the gene flow between species is

represented by the presence of physical, behavioural and genetic barriers that also underlined the boundaries of the different reproductive populations. When barriers to the gene flow evolve between populations as a result of ecologically-based divergent selection, *ecological speciation* occurs (Rundle & Nosil 2005; Schluter 2009; Safran & Nosil 2012). In fact, ecological selection can arise as a consequence of the interaction of individuals with their environment during resource acquisition. This type of speciation differs from other speciation models in which the evolution of reproductive isolation involves key processes other than ecologically-based divergent selection (Rundle & Nosil 2005). In order of understanding the evolutionary process, a fundamental step is to identify in which phase the breakdown caused by barriers occurs and their role in the speciation process. As matter of fact, speciation is a prolonged process with phases in different spatial context but in order to study and classified the different phases, this continuum was dissected in various categories misrepresenting the reality of the natural world (Butlin *et al.* 2008) and the traditional separation of speciation process into allopatric, parapatric and sympatric categories does not capture the complexity of the true spatial relationships that can occur between diverging populations (Butlin *et al.* 2012). In fact, the spatial context is one factor influencing the speciation but, of course, not the only one able to determine the gene flow and it should not be considered a dominant criterion for the classification (Butlin *et al.* 2012). Other factors as a particular habitat and/or the mating time can influence the gene flow between different populations and their speciation rate. It is possible then to cluster the different isolation mechanisms in two major groups, prezygotic and postzygotic and probably considering the former the most effective force in the speciation process known so far. The biological diversity visible today is the direct result of the combined action of speciation, range changes and extinction (Butlin *et al.* 2012) or, shortly, of the evolution. Studying the evolutionary processes involved in generating such vast biodiversity can help us in a better understanding of the patterns followed by populations and species and the dynamics of biodiversity itself.

1.2 The White Continent

Antarctica is a peculiar continent for many different aspects. It is surrounded by the Antarctic Circumpolar Current and therefore isolated not only geographically but also climatically and thermally and oceanographically (Clarke *et al.* 2005; Barnes *et al.* 2006; Griffiths *et al.* 2009; Terauds *et al.* 2012) and presents a unique set of extreme environmental conditions (Walton 1984; Convey 1996a). Because of the extreme conditions together with the Antarctic Treaty System, Antarctica is the only continent without native human populations and it is considered as a land of freedom and cooperation of scientific research. At first sight, Antarctica may appear to be a homogeneous cold continent, but thanks also to recent studies (Terauds *et al.* 2012; Terauds & Lee 2016) sixteen distinct Antarctic Conservations Biogeographic Regions (ACBRs – Figure 1) were identified only within the continent and the Antarctic Peninsula increasing the complexity not only of perceiving the ecosystem but also in the legislation for the conservation measures and protection of the terrestrial habitats and biodiversity across almost all ACBRs (Shaw *et al.* 2014; Hughes *et al.* 2016).

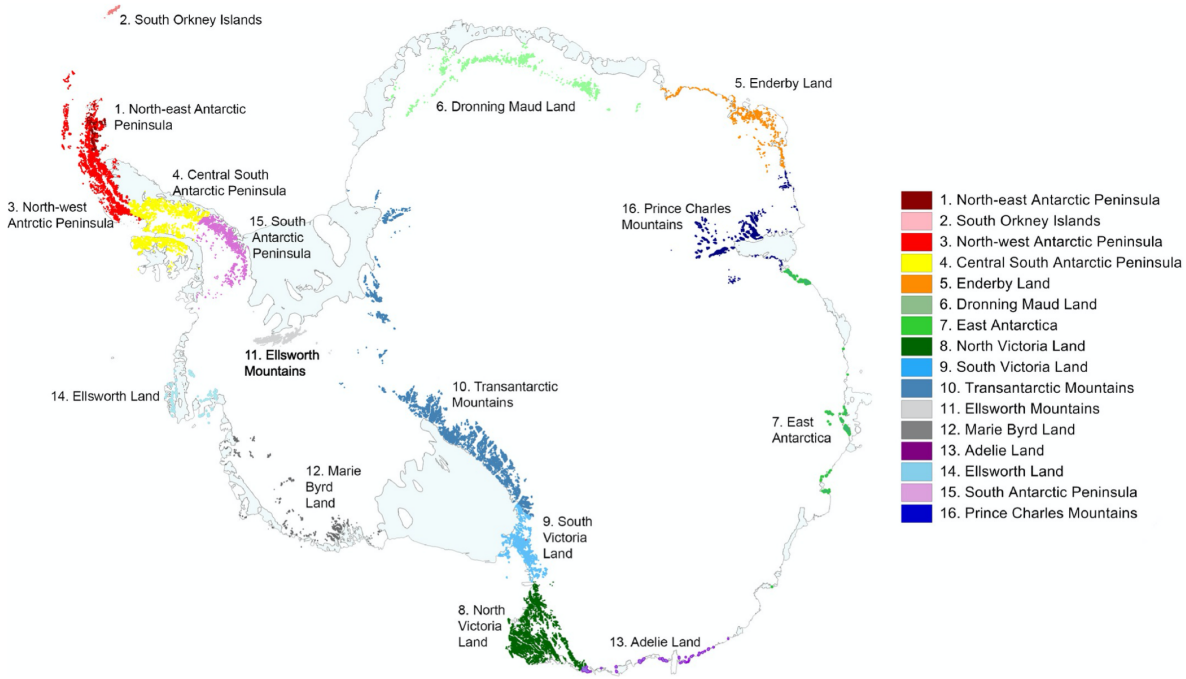

Figure 1. Updated version of the Antarctic Conservation Biogeographic Regions (ACBRs v2 – Terauds & Lee 2016).

Before these recent studies, the Antarctic continent was split in the literature into three main biogeographical and well-defined regions (Figure 2). The sub-Antarctic region includes all the islands and archipelagos surrounding the continent between the Subtropical Front and the Southern Antarctic circumpolar current such as the Marion and Prince Edward Islands, South Georgia, Îles Kerguelen, etc. It is considered the region with the least rigid climate of the entire continent with annual temperatures above the 0°C and precipitation ranges of 2000-3000 mm per year (Bergstrom *et al.* 2006). The maritime Antarctic region includes the remaining islands and archipelagos (South Shetland, South Orkney and South Sandwich archipelagos, Bouvetøya and Peter I Øy islands) and the western part of the Antarctic Peninsula. Although during summer the temperatures are on average above the 0°C, the temperature range between summer and winter is great and the precipitations are scarce (400-500 mm/y; Bergstrom *et al.* 2006). Below the Antarctic Peninsula, Gressitt's ideal line delimits the transition zone with the Continental Antarctic region, based on the differences observed in the composition of the biodiversity of terrestrial organisms (Chown & Convey 2007). This last region is characterized by an extremely rigid climate with temperatures below 0°C even during the summer period and precipitation of about an average of 30-40 mm/year (Bergstrom *et al.* 2006) and the ice covers almost all of the emerged lands, thereby preventing the development of microfauna and flora in practically all the surface with the exception of some areas along the coastline with less rigid temperatures during the summer period and in the Transantarctic Mountains. In fact, the availability of ice-free areas represents a big challenge for the development of the Antarctic's terrestrial ecosystem. The ice-free ground is limited to only 0.34% of the total surface of the continent (about 45000 km^2) and largely restricted to the coastal regions (Convey *et al.* 2009, 2014; Convey 2017). To make things harsher for the Antarctic biotas, the snow-free ground is organized (except for the Dry Valleys region of Victoria Land) in an island-like ecosystem where isolated patches of habitat (nunataks, cliffs, scree slopes, ice-free valleys, coastal oases and islands, ranging in size from less than 1 to thousands of km^2) are surrounded by few meters to many hundreds of kilometres of ice similarly to islands in an ocean (Convey 2010, 2013; Convey *et al.* 2014).

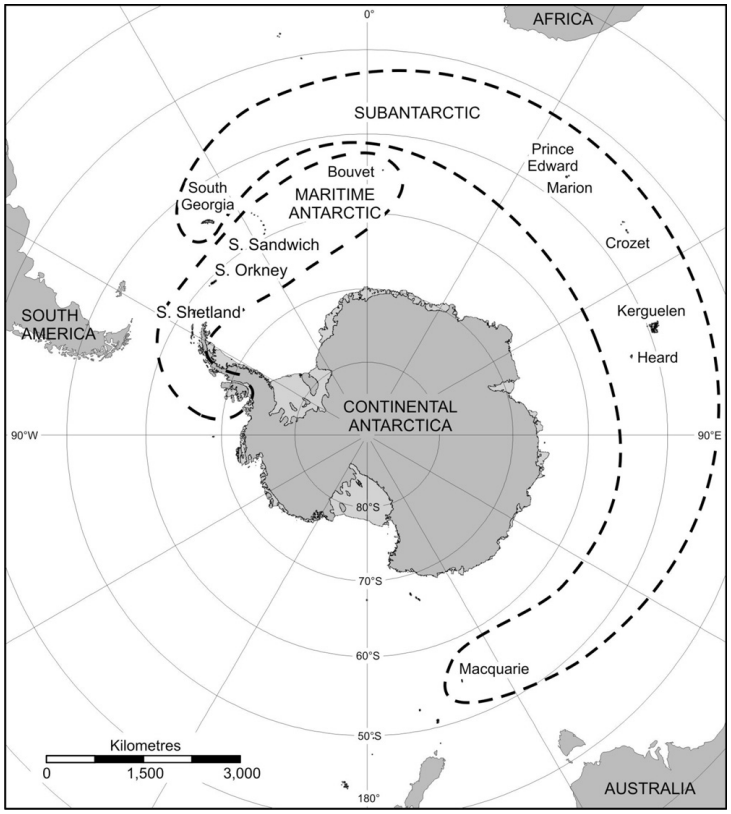

Figure 2. Antarctica's geographical relationship with the other Southern continents and the three commonly described terrestrial biogeographic zones within the Antarctic region (Convey 2013).

Together with the isolation of the habitats, the extreme temperatures, the desiccation, the availability of liquid water and the nutrient limitation created a discontinuous distribution of the invertebrates fauna and the arise of high levels of endemism of many taxa (Convey *et al.* 2008; Pugh & Convey 2008), but also make the Antarctic terrestrial ecosystem vulnerable to any sudden change (Convey *et al.* 2014) that can affect the biological responses on all short-, intermediate- and long-term (i.e. evolutionary) time-scales (Sinclair *et al.* 2003). In fact, the endemic Antarctic terrestrial biota adapted to this highly fragmented ecosystem and its evolution and life-history traits have been mainly regulated by these extreme abiotic factors (Convey *et al.* 2014; Chown *et al.* 2015) while the ecological (i.e. biotic) interactions (e.g. competition, predation) play a minor role in the regulation of the Antarctic terrestrial ecosystem (Convey 1996b; Hogg *et al.* 2006).

Thanks to these peculiar conditions and the few ice-free isolated refugia, the survival of taxa was possible throughout million years and through glacial cycles (Gressitt *et al.* 1963;

Wise 1967; Hogg & Stevens 2002; Stevens & Hogg 2006; Convey & Stevens 2007; McGaughran *et al.* 2008). The populations restricted to the refugia are so exposed to possible divergence in isolation resulting in patterns of morphological and genetic differentiation, eventually with the ultimate potential result of speciation. As a matter of fact, genetic differentiation has been already reported for several Antarctic terrestrial invertebrate populations over both small (< 1 km) and intermediate (from 10 up to 100 km) spatial scales (e.g. Fanciulli *et al.* 2001; Frati *et al.* 2001; Stevens & Hogg 2003; McGaughran *et al.* 2008, 2009). All these abiotic factors, together with the fact that primary producers are restricted to algae, lichens and bryophytes and the largest invertebrates on the soil are represented by microarthropods (Block 1984; Virginia & Wall 1999; Convey 2001), make Antarctica the perfect 'natural laboratory' for the study of the physiological and genetic structure among populations, although remains difficult to predict the biological responses of this delicate terrestrial biota especially on the long-term.

1.3 Terrestrial fauna of Continental Antarctica

Due to the continent's isolation and the extreme environmental factors, the Antarctic terrestrial biota has a limited number of species and many groups are completely missing or very poorly represented (Block 1984; Convey 2001). As a result of the climatic factors, together with the poorly developed soil and apart from few exceptions, lichens and mosses are the only flora able to survive in this habitat, whereas the continental Antarctic fauna consists mainly of few species of terrestrial micro-arthropods (mites and springtails) and other microscopic invertebrates (nematodes, tardigrades and rotifers) (Marshall & Pugh 1996; Convey 2017) making the continental region the simplest ecosystem on earth (Convey 2017). Although this ecosystem appears to be simple in terms of diversity (Block 1984; Convey 2001), in order to survive the harsh Antarctic conditions, the terrestrial organisms have developed biochemical and physiological adaptations that allow them to withstand prolonged periods of both freezing and desiccation (e.g. Sjursen & Sinclair 2002). Together

with biochemical and physiological adaptations, the most common behavioural strategy adopted by springtails (Collembola) and mites (Acari) communities is living under rocks where the environment is usually moist, rich in organic carbon and poor in salinity (Caruso & Bargagli 2007) and where also microbial communities are present and able to stabilizing mineral soils and therefore allowing the colonization by both micro-invertebrates and flora communities as bryophytes, algae and lichens (Convey 2017). Although the temperature plays an important role in the microarthropods' life-cycle, the major factor regulating their survival and growth remains the availability of liquid water (Janetschek 1967; Kennedy 1993). It is possible then, that as a result of the ongoing climate change more habitats will be available for the colonisation of alien species of the terrestrial biota in the future (Sinclair & Stevens 2006) however the change in the mean annual temperature may be associated with changes in extreme climatic conditions (Katz & Brown 1992; Beniston & Stephenson 2004) therefore making any prediction of the response of microarthropods to climate change is difficult. Besides the availability of new niches, a big challenge for microarthropods is represented by their distribution and therefore of their dispersal abilities, especially on the long distances. Although some studies suggested the rafting on the surface of melt-water streams as a possible route for dispersion (Gressitt 1967; Coulson *et al.* 2002; Hawes *et al.* 2008; Hawes 2011) or also through zoohoria (unintentional animal vectors; Pugh 1997) hitch-hiking attached to other (e.g. birds; Falla 1960; although direct bird-mediated transportation has never been demonstrated; Marshall & Pugh 1996) the dispersal by wind may not be effective for microarthropods due to a high risk of desiccation and lack of an anhydrobiotic dispersal stage (Marshall & Pugh 1996; Pugh 2003). In order to understand the dispersion on the short and long distances of the microarthropods inhabiting the area of Victoria Land, molecular studies have been conducted on different springtail species (Fanciulli *et al.* 2001; Frati *et al.* 2001; Stevens & Hogg 2003; Torricelli *et al.* 2010; Carapelli *et al.* 2020). The presence of glacial barriers in Victoria Land greatly affected the distribution of the terrestrial microarthropods probably limiting the gene flow to restricted and isolated refugia during the Last Glacial Maximum (Frati *et al.* 2001; Stevens & Hogg 2003; Stevens *et al.* 2006). The same biogeographical patterns along Victoria Land appear to be generally similar also for

the Prostigmata mite *Stereotydeus mollis* (Stevens & Hogg 2006; McGaughran *et al.* 2008, 2010; Demetras *et al.* 2010) although with higher genetic divergence, probably due to the smaller size, higher activity levels and shorter generation time (Martin & Palumbi 1993) and/or to the speciation processes occurred through a greater number of glacial cycles (more than 10 Myr) than for the springtails and still ongoing nowadays. Because the colonization, the adaptation and the survival of these microarthropods on the Antarctic continent were accomplished over thousands of years, they represent the suitable taxa to test speciation hypothesis and evolutionary trends and history of the Antarctic fauna (Sinclair & Stevens 2006; Stevens & Hogg 2006).

1.4 A free-living mite of Antarctica: Stereotydeus Berlese, 1860

Because of their versatility and ability to occupy the most unfavourable type of soils and environments, the free-living mites are the most abundant and widespread group in Antarctica (Gressitt & Shoup 1967). Among the free-living Antarctic mites, the most represented groups are the Prostigmata, the Oribatida and the Mesostigmata and within the Continental and Maritime Antarctica 40 different species have been recorded considering only the two first order with 12 genera from 6 families for the Prostigmata and 8 genera from 7 families for the Oribatida (Marshall & Pugh 1996). In particular, within the Prostigmata, one of the most represented families is certainly the Penthalodidae with the cosmopolitan genus *Stereotydeus* Berlese, 1860 (Marshall & Pugh 1996). Until now, eight *Stereotydeus* species have been recorded for Continental and Maritime Antarctica and of these, three (*S. delicatus* Strandtmann, 1967, *S. punctatus* Strandtmann, 1967 and *S. belli* (Trouessart, 1902)) are known from North Victoria Land and two (*S. mollis* Womersley & Strandtmann, 1963 and *S. shoupi* Strandtmann, 1967) from South Victoria Land and the immediate vicinity and the central Transantarctic Mountains (Table 1), probably occurring with no detected overlap (Demetras *et al.* 2010).

Table 1. List of *Stereotydeus* species, currently known from Antarctica, their distribution ranges and references associated with each species.

Penthalodidae	Lat. (S)	Long.	ACBRs[1] / Area	References
S. delicatus Strandtmann, 1967	66° – 72°23′	163°E – 170°13′E	North Victoria Land	Strandtmann 1967
S. punctatus Strandtmann, 1967	71°18′ – 72°40′	169°25′E – 170°13′E	North Victoria Land	Strandtmann 1967; Gressitt & Shoup 1967; Fredes 2015
S. belli (Trouessart, 1902) [2]	71° – 74°04′	165°18′E – 172°E	North Victoria Land	Womersley & Strandtmann 1963; Gressitt & Shoup 1967; Strandtmann 1967; Stevens & Hogg 2006; Caruso & Bargagli 2007
S. mollis Womersley & Strandtmann, 1963 [3]	74° – 78°05′	161°E – 168°E	South Victoria Land, Transantarctic Mountains	Womersley & Strandtmann 1963; Strandtmann 1967; Fitzsimons 1971; Pittard 1971; Pittard *et al.* 1971; Strandtmann & George 1973; Block 1985; Sinclair & Sjursen 2001; Sjursen & Sinclair 2002; Stevens & Hogg 2002, 2006; Caruso & Bargagli 2007; McGaughran *et al.* 2008; Demetras *et al.* 2010
S. shoupi Strandtmann, 1967	79°52′ – 84°73′	159°9′E– 176°29′W	South Victoria Land, Transantarctic Mountains	Strandtmann 1967; Stevens & Hogg 2006; Demetras *et al.* 2010
S. meyeri Strandtmann, 1967	67°30′ – ?	46°E – ?	Enderby Land	Strandtmann 1967
S. villosus (Trouessart, 1902)	60°35′ – 71°50′	68°30′W– 45°30′W	North-west Antarctic Peninsula, South Shetland Islands, South Orkneys Islands	Womersley & Strandtmann 1963; Strandtmann 1967; Wallwork 1973; Graham 1975; Convey & Smith 1997; Pugh & Convey 2000; Stevens & Hogg 2006
S. intermedius Trouessart, 1907 [4]	60°35′ – ?	45°30′W– ?	South Orkneys Islands	Strandtmann 1967; Strandtmann 1970; Wallwork 1973; Pugh & Convey 2000; Fredes 2015
S. reticulatus Strandtmann, 1970	54°0′ – 54°32′	36°0′W– 38°3′W	South Georgia *(Sub Antarctic Island)*	Strandtmann 1970; Wallwork 1973
S. longipes Strandtmann, 1970	54°0′ – 54°32′	36°0′W– 38°3′W	South Georgia *(Sub Antarctic Island)*	Strandtmann 1970; Wallwork 1973; Pugh 1993; Qin 1998; Fredes 2015

[1] see **Figure 1**.

[2] a larger form of *S. belli* was recorded on Possession Island only (Gressitt & Shoup 1967).

[3] The northern and southern distributional limits of *S. mollis* are uncertain due to the very limited sampling; only 1 adult female from Cape Adare and 2 adult females from Camp Plateau (Womersley & Strandtmann 1963).

[4] after the first description by Trouessart 1907 no other information of this species is given; in a note, Strandtmann (1970 p. 98) suggests a similarity with *S. longipes* but concludes that more samples are needed from South Orkneys Island to confirm or not the description.

While many studies have been conducted on the morphological and genetical characteristics of the Antarctic springtails (see Fanciulli *et al.* 2001; Frati *et al.* 2001; McGaughran *et al.* 2010; Torricelli *et al.* 2010; Greenslade 2018a; Greenslade 2018b; Carapelli *et al.* 2020) present along Victoria land, very few investigate the biodiversity of the Antarctic mites, especially for the genus *Stereotydeus*. In fact, after the first morphological studies in the 1960s (Womersley & Strandtmann 1963; Strandtmann 1967; Pittard 1971), few works on the physiology and ecology of the genus were conducted (see for examples Gressitt & Shoup 1967; Fitzsimons 1971; Block 1985; Sinclair & Sjursen 2001; Sjursen & Sinclair 2002; Caruso & Bargagli 2007) with particular attention to *S. mollis*. Exclusively on this latter species, in the last twenty years, only three genetic studies (Stevens & Hogg 2006; McGaughran *et al.* 2008; Demetras *et al.* 2010) were conducted in the Southern Victoria Land, giving us a taste of the high level of diversity that this species alone can hide within and among different populations and its evolutionary history and relationships to other species.

Because of the harsh field conditions and the small size of the specimens of the genus, the exact determination of the species in situ is impossible, but once in the laboratory, the combination of the genetical and morphological approaches can be a powerful tool capable of detecting different levels of diversity. During the last two decades, the development of the barcoding techniques using the mitochondrial cytochrome *c* oxidase subunit I (*cox1*) gene in combination with different nuclear markers, helped us discriminating cryptic (morphologically similar) species and determining the origin of the morphological variation. But this cannot be successfully done without a previous solid morphological analysis able to discriminate, at the higher taxonomic ranks, the broad location of the specimens on the phylogenetic tree. Especially with such small and variable animals living in such isolated and harsh conditions, it is important that two powerful tools (i.e. the morphological and genetical approaches) are combined to allow the drawing of lines in the identification and characterization of the species, thereby laying the foundations for future decisions in conservation matters concerning Antarctica's unique terrestrial fauna.

The delicate balance and stability of ecosystems and consequently of biodiversity, are facing now the consequences of various threats as environmental changes and biological invasions (Frenot *et al.* 2005). Especially for remote and isolated ecosystems, such as the polar regions, the knowledge of the biological processes and the biodiversity is still incomplete. In order to take action and counter the impact of these changes on such delicate ecosystems, a fundamental step is to study their resilience to disturbance (Convey & Peck 2019). Despite the general idea of Antarctica as a pristine continent, unfortunately, fairly recent studies shown that also the Antarctic ecosystem and biodiversity are facing the same threats of the rest of the world and especially from climate change, pollution, biological invasions and the rising of human activities (Chown *et al.* 2012; Lee *et al.* 2017; Bergami *et al.* 2020) and the poor knowledge of species diversity and of their dispersal ability are considered limiting factors that prevent the implementation of sustainable conservation plans (Carapelli *et al.* 2017, 2020; Wauchope *et al.* 2019).

1.5 Aims

Considering the limited information available regarding the distribution of the *Stereotydeus* genus in Victoria Land, with this study I wanted to investigate the genetic and morphological variability of this genus through population genetics and morphological approach. Previous studies have shown a considerable haplotype variety among the populations of the Antarctic mite *S. mollis* but, apart from generic ecological studies in North Victoria Land on terrestrial arthropods (Caruso & Bargagli 2007), nothing is known about the *Stereotydeus* distribution and diversity within the area that could be considered the contact point between the two species *S. mollis* and *S. belli* (Stevens & Hogg 2006; McGaughran *et al.* 2008; Demetras *et al.* 2010). Therefore, in order to study population genetics, molecular systematics and the morphology of this genus, for this work, I used DNA extracted from *Stereotydeus* spp. sampled in 9 different populations of both North and South Victoria Land (Figure 3; Table 2). The current populations' patterns and distribution and their genetic structure can be the results of several processes as gene flow, genetic drift

and fragmentation. In particular, repeated glacial events have likely had a pronounced influence on the present species ranges and population sizes both directly (through natural selection) and indirectly (through their effect on individuals and habitat and refugial availability (e.g Rowe *et al.* 2004). Populations that become restricted to such refugia may undergo divergence in isolation for instance through changes in population size, founder and bottlenecks events followed by genetic drift. For a population, the chances of experiencing variation in its gene pool are indirectly related to the number of individuals itself, so when small populations start differentiation processes it is not unlikely that an increase in the genetic differences between populations will result in speciation. The ongoing climate changes are affecting also the Antarctic continent through modification of the environmental conditions and eventually of the food webs and interactions between co-evolved species, altering the delicate balance of this peculiar ecosystem (Convey 2001; Nielsen & Wall 2013). Therefore, a more accurate and in-depth study of the endemic species of the Continental Antarctic ecosystem can help us understand the possible developments of future ecological changes and be an added value for the fulfilment of the Antarctic conservation plans.

2. MATERIALS AND METHODS

2.1 Samples collection

During the Antarctic summer expeditions of 2017-2018 and 2018-2019 of the Italian National Antarctic Research Program (PNRA: PNRA16_00234), several *Stereotydeus* specimens were collected from 9 different localities of Victoria Land (Figure 3; Table 2) and preserved in absolute ethanol. The samples intended for the genetic analyses were stored at -80°C, whereas the stock for the morphological identification and SEM preparation was preserved at room temperature.

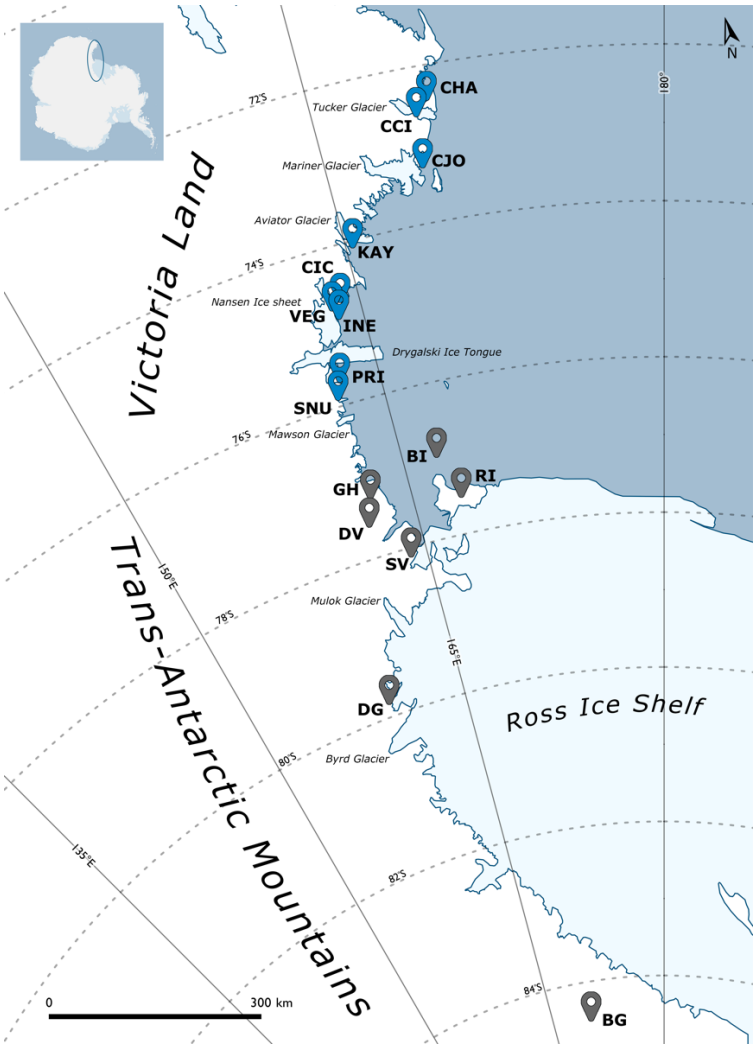

Figure 3. Map of sampling localities for the new *Stereotydeus* spp. samples analysed for this study (blue) and from *S. mollis* and *S. shoupi* Stevens & Hogg 2006; McGaughran *et al.* 2008; Demetras *et al.* 2010 (grey): DV= McMurdo Dry Valleys (Taylor, Wright and Victoria valleys and vicinity), SV = Southern Dry Valleys (Garwood, Marshall and Miers valleys and vicinity), BI = Beaufort Island; RI = Ross Island and GH = Granite Harbour.

Table 2. Coordinates and altitudes of sampling sites and ID codes for different populations and number of individuals obtained for the preliminary analyses (**n.**). *CIC, VEG and INE have been arbitrarily considered as "Central" to facilitate the division of the sampling area although they should be formally considered part of the ACBRs of North Victoria Land.

ID	Locality	Lat (S)	Long (E)	Victoria Land	Altitude	n.
CHA	*Cape Hallett (Adelie Cove)*	72°26′25″	169°56′32″	North	140 m	10
CCI	*Crater Cirque*	72°37′52″	169°22′22″	North	200 m	14
CJO	*Cape Jones*	73°16′38″	169°12′54″	North	310 m	10
KAY	*Kay Island*	74°04′14″	165°18′60″	North	140 m	10
CIC	*Campo Icaro*	74°42′45″	164°06′21″	Central*	70 m	32
VEG	*Vegetation Island*	74°47′00″	163°37′00″	Central*	120 m	10
INE	*Inexpressible Island*	74°53′39″	163°43′44″	Central*	30 m	10
PRI	*Prior Island*	75°41′31″	162°52′34″	South	130 m	15
SNU	*Starr Nunatak*	75°53′57″	162°35′08″	South	60 m	10

2.2 Preliminary DNA extraction, amplification and sequencing

Total genomic DNA was extracted from a total of 121 individuals from the 9 sampled populations (Table 2) using the Wizard® SV genomic DNA Purification System (Promega, Madison, WI, USA) and used for PCR amplifications. The region II of the mitochondrial cytochrome *c* oxidase I (*cox1*) gene was amplified using the specific primers Mite-COI-2F (5′-TTYGAYCCIDYIGGRGGAGGAGATCC-3′) and Mite-COI-2R (5′-GGRTARTCWGARTAWCGNCGWGGTAT-3′) (Otto & Wilson 2001). In order to amplify the 28S gene, a preliminary amplification was performed on a restricted pool of five individuals per six different populations with the primer pairs D1a (5′-CCCSCGTAAYTTAAGCATAT-3′) and D5b1 (5′-ACACACTCCTTAGCGGA-3′). For each amplification, the total reaction volume of 25 μl contained 2.5 μl of genomic DNA, 0.5 mM of each primer, 0.2 mM of each deoxyribonucleotide triphosphates (dNTPs), 2.5 mM of $MgCl_2$, 5 μl of Green GoTaq Flexi buffer and 0.625 U of GoTaq Flexi DNA Polymerase (Promega, Madison, WI, USA). The amplifications were performed in a GeneAmp® PCR System 2700 (Applied Biosystems, Foster City, CA, USA) thermal cycler. The initial denaturation step was set at 95°C for 5 min, followed by 35 cycles at 95°C for 1 min, 50°or 45°C (for the *cox1*) and 50°C (for the *28S*) for 1 min and 72°C for 90 s and a final extension step at 72°C for 7 min.

The sequences obtained were aligned using the MacVector software (version 16.0.8 - MacVector, Inc. 2018) and new primers were identified in conserved regions of the *28S* fragment. A 750 bp of the large ribosomal subunit was then amplified for the 121 specimens with the new specific primer pair Ste-28S-F (5'-GGACGTGAAACCGCTTGTA-3') and Ste-28S-R (5'-TCTGACGATCGATTTGCAC-3') following the above-mentioned cycling conditions, but with the annealing temperature set at 50°C. The PCR products were then purified using the kit Wizard® SV Gel and PCR Clean-up (Promega, Madison, WI, USA) and sequenced on both strands (with the same primers used for PCRs) with a DNA Analyzer ABI 3730, at the core facility of the Bio-Fab Research Lab (Rome, Italy). The sequences were assembled and manually corrected using the MacVector software (version 16.0.8 - MacVector, Inc. 2018).

2.3 Assembling the preliminary dataset and pointing out some discrepancies

In addition to the new samples extracted for this study, other 56 reference *cox1* sequences from different *Stereotydeus* species already published were downloaded from GenBank (Table A in Appendix) and included in the analyses: 50 of *S. mollis* (Accession numbers DQ305368, DQ305385-DQ305387, DQ305389-DQ305398, DQ309572-DQ309574 (Stevens & Hogg 2006); DQ305361-DQ305365, DQ305367, DQ305369-DQ305384 (McGaughran *et al.* 2008); HM537082-HM537092 (Demetras *et al.* 2010)), two sequences of *S. shoupi* (Accession numbers: DQ309575, DQ309576), one of *S. belli* (Accession number: DQ309577), one of *S. villosus* (Accession number: DQ309578) and two of *Stereotydeus* sp. (Accession number: DQ309579, DQ309580) together with 2 outgroups: the winter grain mite *Penthaleus major* (Acari: Penthaleidae; under submission, Accession number not yet assigned) and another eupodine mite *Eriorhynchus* sp. (Acari: Eriorhynchidae; Accession number AF142135; see Appendix, Table A).

The two haplotypes DQ305366 (S2 - McGaughran *et al.* 2008), DQ305388 (B - Stevens & Hogg 2006) were excluded from this analysis because they are homonyms of DQ305362,

DQ305389 respectively. Probably, an error in naming them occurred while were being deposited on GenBank therefore, following the analyses of Demetras *et al.* (2010), I used the latter two. Although in this analysis I included all the remaining haplotypes deposited, I need to highlight some incongruences in other three sequences: *i)* for DQ305362 (S2 - McGaughran *et al.* 2008) coordinates are missing because is not clear the exact sampling site of the Wright Valley (W3 and/or W5); *ii)* for DQ305382 (S20 – V11 from Victoria Valley; McGaughran *et al.* 2008) coordinates are not indicated because they are missing in the original article (Table 1, McGaughran *et al.* 2008); *iii)* DQ305367 (S6 - McGaughran *et al.* 2008) has been used in Demetras *et al.* (2010) but is missing in the original article of McGaughran *et al.* (2008), therefore the coordinates are not shown (see Table A in the Appendix). For specimens from Demetras *et al.* (2010) only the generic location of southern Dry Valleys (i.e., Garwood, Marshall and Miers Valleys, Shangri La (according to Collins *et al.* (2019) and vicinity) was given but not the exact coordinates so they are not shown in this study.

Sequences of the *cox1* dataset were aligned using the online tool Clustal Omega (Sievers *et al.* 2011) and manually corrected and trimmed using the MacVector software (version 16.0.8 - MacVector, Inc. 2018).

A first alignment of the *28S* dataset was also performed on the sequences of the extracted individuals using the online tool Clustal Omega (Sievers *et al.* 2011; available at https://www.ebi.ac.uk/Tools/msa/clustalo/) and then was manually corrected and trimmed using the MacVector software (version 16.0.8 - MacVector, Inc. 2018). In order to remove poorly aligned positions and divergent regions, the hyper-variable regions of the *28S* alignment were discarded using the online tool Gblocks server 0.91b (Castresana 2000; available at http://molevol.cmima.csic.es/castresana/Gblocks_server.html) selecting strict combination settings and removing from the alignment also the contiguous nonconserved positions (i.e., all gap/missing bases in at least one sequence were removed). The 97% of the initial *28S* dataset was conserved and concatenated to the *cox1* alignment through FaBox (Villesen 2007) with the online tool Fasta alignment joiner (available at https://users-birc.au.dk/palle/php/fabox/alignment_joiner.php).

2.4 Preliminary phylogenetic analyses

The dataset, assembled as described before, was partitioned in four different charsets: 1st, 2nd and 3rd codon positions for *cox1* and the *28S* region; the software PartitionFinder 2.1.1 (Lanfear *et al.* 2016) was applied to find the evolutionary models that better fit our dataset. The choice spanned from simpler models that take into account the free exchangeability of all parameters (GTR) to more complex once that consider the nucleotides position to vary differently among the site rate variation and the frequency of all possible transformation of one base to every other (e.g. TRN; see Table 5). Once the best evolutionary model was selected, it was applied for the reconstruction of the phylogenetic tree carried out with the MrBayes 3.2.7 software (Ronquist *et al.* 2012). The Bayesian analysis was then performed applying four chains for 10^6 generations, with a sampling frequency of one tree every 1000 iterations and with the 25% of the trees discarded (burn-in step) from the final result. The resulting phylogenetic tree was then visualized with the FigTree 1.4.4 software (Rambaut 2012).

2.5 Preliminary morphological analyses

After this first preliminary genetic screening, it was immediately evident that some of the resulting sequences were too diverse from the rest of the haplotypes already deposited on GenBank by Stevens & Hogg 2006, McGaughran *et al.* 2008 and Demetras *et al.* 2010 and morphological approach was therefore indispensable to proceed any further.

A first morphological screening was conducted on a few specimens from all the population, except for VEG where the samples for the morphology were not available (Table 3), in order to save the remaining individuals for the scanning electron microscope (SEM) preparation and future analyses. The *Stereotydeus* specimens were incubated into a slide with few drops of lactic acid (20%) at room temperature for three weeks allowing the samples to be cleared up. Samples were then observed under a Leica DM RBE microscope.

After the observation under the optical microscope, some specimens were prepared for SEM visualization (Table 3). A first step in absolute ethanol was necessary to clean the samples and dehydrate them. Then, the samples were dried out of the ethanol in a Balzer CPD 010 reaching the CO_2 critical-point. Samples were fixed on an aluminium stub and then coated with gold-palladium in a Balzer MED 010 and observed with a Philips XL20 electron microscope.

Table 3. Sampling localities with respective area codes and coordinates, the year of the Antarctic expedition during which the samples were collected, the date of collection, the number of the preliminary specimens prepared for optical microscopy (n.) and scanning electron microscopy (SEM).

Area	ID Area	Coordinates	Expedition	Date	n.	SEM
Cape Hallett	CHA	72°26'25"S 169°56'32"E	2018-2019	9/01/2019	19	4
Crater Cirque	CCI	72°37'52"S 169°22'22"E	2018-2019	9/01/2019	6	-
Cape Jones	CJO	73°16'38"S 169°12'54"E	2017-2018	29/12/2017	2	-
Kay Island	KAY	74°04'14"S 165°18'60"E	2017-2018	26/12/2017	1	-
			2018-2019	16/01/2019	17	
			2018-2019	27/01/2019	6	
Campo Icaro	CIC	74°42'45"S 164°06'21"E	2017-2018	24/12/17	9	4
			2018-2019	28/01/19	14	5
Vegetation Island	VEG	74°47'22"S 163°38'09"E	No samples for microscopy		-	-
Inexpressible Island	INE	74°53'39"S 163°43'44"E	2018-2019	21/01/2019	10	6
Prior Island	PRI	75°41'31"S 162°52'34"E	2017-2018	11/01/18	10	
			2018-2019	11/01/19	12	10
Starr Nunatak	SNU	75°53'57"S 162°35'08"E	2017-2018	11/01/18	-	5
			2018-2019	11/01/19	4	-

2.6 New molecular and morphological preparations

In order to work backward in linking the specific molecular data to the morphology of the specimens, an additional preparation of new individuals was necessary after the screenings. The individuals for the new analyses were carefully chosen from 5 populations (CJO, CIC, INE, PRI and SNU) preserved at -80°C. From 5 up to 13 specimens each population were used for the analyses (Table 4).

This time, the DNA extraction was performed following the above-mentioned protocol only on 2-4 legs of each animal, while the rest of the body was incubated into a slide with few drops of lactic acid (20%) at 37-45 °C for 30 minutes allowing the samples to be cleared up and then were observed under a Leica DM RBE microscope.

Table 4. New specimens extracted for the haplotypic and morphological analyses. Sampling localities with the date of collection, the ID and the life-cycle stage (nymph / adult) of the new *Stereotydeus* individuals.

AREA	Year	ID	Stage	AREA	Year	ID	Stage
Cape Jones	29/12/17	J1	nymph	*Starr Nunatak*	11/01/18	S1	adult (M)
		J2	nymph			S2	adult (M)
		J3	nymph			S3	nymph
		J4	nymph			S4	nymph
		J5	nymph			S5	adult (F)
		J6	adult (F)			S6	adult (F)
		J7	adult (M)			S7	adult (F)
Campo Icaro	28/01/19	CI1	adult (M)	*Prior Island*	11/01/19	P1	adult (M)
		CI3	adult (F)			P2	adult (M)
		CI4	nymph			P3	adult (F)
		CI5	adult (F)			P5	adult (M)
		CI6	nymph			P6	adult (F)
		CI7	adult (M)				
		CI8	nymph			P7	adult (F)
	24/12/17	CI9	adult (M)	*Inexpressible Island*	21/01/19	I1	adult (F)
		CI10	adult (M)			I2	adult (M)
		CI11	adult (F)			I3	adult (F)
		CI12	adult (F)			I4	adult (M)
		CI13	adult (F)			I5	adult (F)
		CI14	adult (M)				

The *cox1* and the *28S* of the new samples were then amplified following the PCR protocols and cycling conditions mentioned above. The PCR products were then purified using the kit Wizard® SV Gel and PCR Clean-up (Promega, Madison, WI, USA) and sequenced on both strands, using the same primers applied for PCRs, with a DNA Analyzer ABI 3730, at the core facility of the Bio-Fab Research Lab (Rome, Italy). The sequences were then assembled and manually corrected using the MacVector software (version 16.0.8 - MacVector, Inc.). The sequences of both *cox1* and *28S* new datasets were aligned together with the previous matrix of data using the online tool Clustal Omega (Sievers *et al.* 2011; available at https://www.ebi.ac.uk/Tools/msa/clustalo/) and manually corrected and trimmed using the MacVector software (version 16.0.8 - MacVector, Inc.). The resulting *cox1* dataset was aligned then with the two outgroups, while the *28S* dataset only with the *P. major* outgroup due to the lack of the ribosomal DNA sequence for *Eriorhynchus* sp. Since the *28S* of *Eriorhinchus* sp. was not available on the Genbank server, only *P. major* was used as an outgroup and the *cox1* dataset was concatenated to the *28S* alignment into a multi-locus dataset through FaBox (Villesen 2007) with the online tool Fasta alignment joiner. Then, the multi-locus alignment was run on the Gblocks server 0.91b (Castresana 2000;

available at http://molevol.cmima.csic.es/castresana/Gblocks_server.html) under strict settings conditions and the hyper-variable regions of the *28S* alignment were discarded. After the run, 1,034 positions out of the 1,171 of the initial dataset (88%) were kept.

At the end of the preparation, the six single- and the multi-locus alignments used for the phylogenetic and the population genetics analyses were: *i) cox1* unrooted; *ii) 28S* unrooted; *iii) cox1* rooted; *iv) cox1* haplotypes; *v)* combined; *vi)* combined morphology (Table 5).

Table 5. List of the dataset (single and multi-locus), markers, reference sequences and outgroups used for the analyses and models of nucleotide evolution that best fit our datasets, divided according to the partition applied and to the respective tree search optimization criteria.

		n.	*cox1*	*28S*	Ref.	Outgroups	Best model			
							1st	2nd	3rd	non-cod.
i	***cox1 unrooted***	159	x	-	*S. shoupi (2)* *S. villosus* *Stereotydeus sp. (2)* *S. belli*	-	TRN+G	TRN+I	F81+I	-
ii	***28S unrooted***	159	-	x	-	-	-	-	-	HKY+I
iii	***cox1 rooted***	159	x	-	*S. shoupi (2)* *S. villosus* *Stereotydeus sp. (2)* *S. belli*	*Eriorhynchus sp.* *P. major*	K81UF+I+G	GTR+I	F81+I	-
iv	***cox1 haplotypes***	159	x	-	*S. shoupi (2)* *S. villosus* *Stereotydeus sp. (2)* *S. belli* *S. mollis (50)*	*Eriorhynchus sp.* *P. major*	K81UF+G	GTR+I+G	F81+I	-
v	***combined***	159	x	x	-	*P. major*	K81UF+I+G	TRN+I	F81+I	GTR+G
vi	***combined morphology***	99	x	x	-	*P. major*	HKY+I+G	TIM+G	F81+G	TVM+G

2.7 Phylogenetic analyses

In order to identify the haplotypes and their frequency within populations, all the alignments were run with the online software DNA-Collapser (Villesen 2007; available at https://users-birc.au.dk/palle/php/fabox/dnacollapser.php). The sequences of the resulting haplotypes were used to calculate the genetic distances between the haplotypes using the software R 3.6.1 (R Core Team 2016) with the "ape" package (Paradis & Schliep 2019) (Table 8). The best evolutionary models were selected before the tree search (Table 5), partitioning the datasets with the software PartitionFinder 2.1.1 (Lanfear *et al.* 2016) based on the Akaike's information criterion (AIC) and a greedy strategy: 1st, 2nd and 3rd codon positions

for the *cox1* protein-encoding gene and one single partition was considered for the *28S* (Table 5). The Bayesian analysis was performed with the MrBayes 3.2.7 software (Ronquist *et al.* 2012) applying four chains (three hot and one cold) for 10^6 generations, with a sampling frequency of one tree every 1000 iterations and with 25% of the tree topologies discarded (burn-in step) from the final result. For a better visualization, the resulting phylogenetic trees were then modified with the FigTree 1.4.4 software (Rambaut 2012). After the positive identification of the specimens, the new *Stereotydeus* mitochondrial and the nuclear sequences were deposited in GenBank (under submission, Accession number not yet assigned).

2.8 Population structure analysis

The population genetics study was performed using the *cox1* unrooted dataset applied for the phylogenetic analysis. Haplotype frequencies were obtained using the online tool DNAcollapser (Villesen 2007; available at https://users-birc.au.dk/palle/php/fabox/dnacollapser.php). The network clade analysis was performed on TCS 1.21 (Clement *et al.* 2000) using the connection limit of 98% and visualized with the online tool tcsBU (Múrias dos Santos *et al.* 2016; available at https://cibio.up.pt/software/tcsBU/) to estimate the haplotype networks for each species. To investigate the genetic characteristics of populations and to test for the presence of population structure, the software Arlequin version 3.11 (Excoffier *et al.* 2005) was used for each species separately. The haplotype (h) and nucleotide (π) diversity indices (Nei 1987), as well as the mean number of pairwise differences (θ) and segregating sites (θS), were computed at the population levels. Analysis of molecular variance (AMOVA; Excoffier *et al.* 1992) was used to measure the extent to which genetic variance could be assigned to the hierarchical structure of population organization (testing them with the structure according to the populations: 'Cape Hallett', 'Crater Cirque', 'Cape Jones' and 'Kay Island' for *S. belli*; 'Campo Icaro', 'Vegetation Island' and 'Inexpressible Island' for *S. delicatus*; 'Campo Icaro', 'Inexpressible Island', 'Prior Island' and 'Starr Nunatak' for *S. ineffabilis* sp. nov. (see Results

– *3.1.2 Morphological Description*) and 'Prior Island' and 'Starr Nunatak' for *S. nunatakis* sp. nov. (see Results – *3.1.2 Morphological Description*)), with the statistical significance of variance components tested with 16,000 permutations. Pairwise differences between haplotypes (Φ_{ST} values) were calculated using simple distances and these were used to look for significant relationships between population genetic distance (Φ_{ST}).

3. RESULTS

In this section, I will provide the description of two novel species of *Stereotydeus* followed by the molecular results. The identification was possible thanks to the strong connection of molecular and morphological evaluation. In fact, through the parallel comparison of the morphology of the individuals and the molecular data, it was possible to identify well-defined characteristics in both the body aspects than in the genetical sequences that allowed a clear separation of the two novel species from the other *Stereotydeus* taxa already described in the previous works. In order to better understand the following phylogenetic analyses, I will provide first the full description of the two new *Stereotydeus* species and I will discuss the molecular aspects afterwards.

3.1 Morphological study

3.1.1 Systematics

Characteristics used to identify the genus *Stereotydeus* are the presence of a trilobed epirostrum with epivertex at the base of the middle lobe, genitalia in a nearly circular camerostome covered by two flaps separated from the body by a distinct suture (Strandtmann 1967). On the species level, the characteristics used to separate the different species are the division in two segments of the femora, the degree of development of the epirostrum and dorsal sculpturing. In addition to the above-mentioned characteristics, the following features were useful in distinguishing our species from those already described: body length, the position of solenidia, length of the apical (4^{th}) segment of pedipalps and length of the movable arms of the chelicerae, presence of the rhagidial organs on the tibiae, the symmetry or asymmetry of the rhagidiform organs on the tarsi and the shape of the apical setae on top of the tarsi, numbers of the aggenital setae and the position of the anal pore on the hysterosoma.

3.1.2 Morphological Description

- ***Stereotydeus ineffabilis*** sp. nov.: Brunetti and Siepel.

Type Locality: Inexpressible Island (74°53'39"S 163°43'44"E), Central Victoria Land, Continental Antarctica.
Holotype: slide STI1, male, 21 January 2019. Deposited at the Collection of the Department of Life Sciences at the University of Siena.
Paratypes: STI2, male, Prior Island (75°41'31"S 162°52'34"E), South Victoria Land, Continental Antarctica, 11 January 2019; STI3_I2, male, Inexpressible Island, 21 January 2019; STI4, male, Inexpressible Island, 21 January 2019; STI5, male, Prior Island, 11 January 2019. Deposited at the Collection of the Department of Life Sciences at the University of Siena.

Material Examined for the Description

Prior Island, 2+6 slides (the former number indicates slides with multiple individuals while the latter number indicates slides with just a single individual) (10+1♀, 10+5♂, 3+0 nymphs); Inexpressible Island, 1+6 slides (6+3♀, 3+3♂); Campo Icaro (74°42'45"S 164°06'21"E), Central Victoria Land, Continental Antarctica, 1+2 slides (2+1♀, 3+0♂, 4+1 nymphs); Starr Nunatak (75°53'57"S 162°35'08"E), South Victoria Land, Continental Antarctica, 3 slides (1♀, 2♂). The specimens will be conserved at the Collection of the Department of Life Sciences at the University of Siena.

Etymology: From Latin, *ineffabilis* meaning inexpressible, ineffable because of its fragile nature.

Description: Soft-bodied mite with a barely visible sculptured pattern on the prodorsum and sclerotization almost absent. The body length of the Holotype is 408,44 μm; the average length of adult specimens studied is 414 μm, with values ranging from 369 to 460 μm (± 25 μm). The size is smaller than *S. mollis* (Womersley & Strandtmann 1963; Pittard 1971). The shape of the body is similar to the other species of the genus. The propodosoma is divided from the hysterosoma by a distinct sejugal furrow.

Dorsal side: The epirostrum is trilobed but weakly developed with a slightly striated epivertex with two ciliated setae at the base of the middle lobe (Figures 4a and 8c). The eyes are convex and lightly striated. Three pairs of slit pores are present on each side of the dorsum (Figures 4a and 8a); as for other species of the genus, the hysterosoma carries 8 pairs of plumose setae (Figures 4a and 8a).

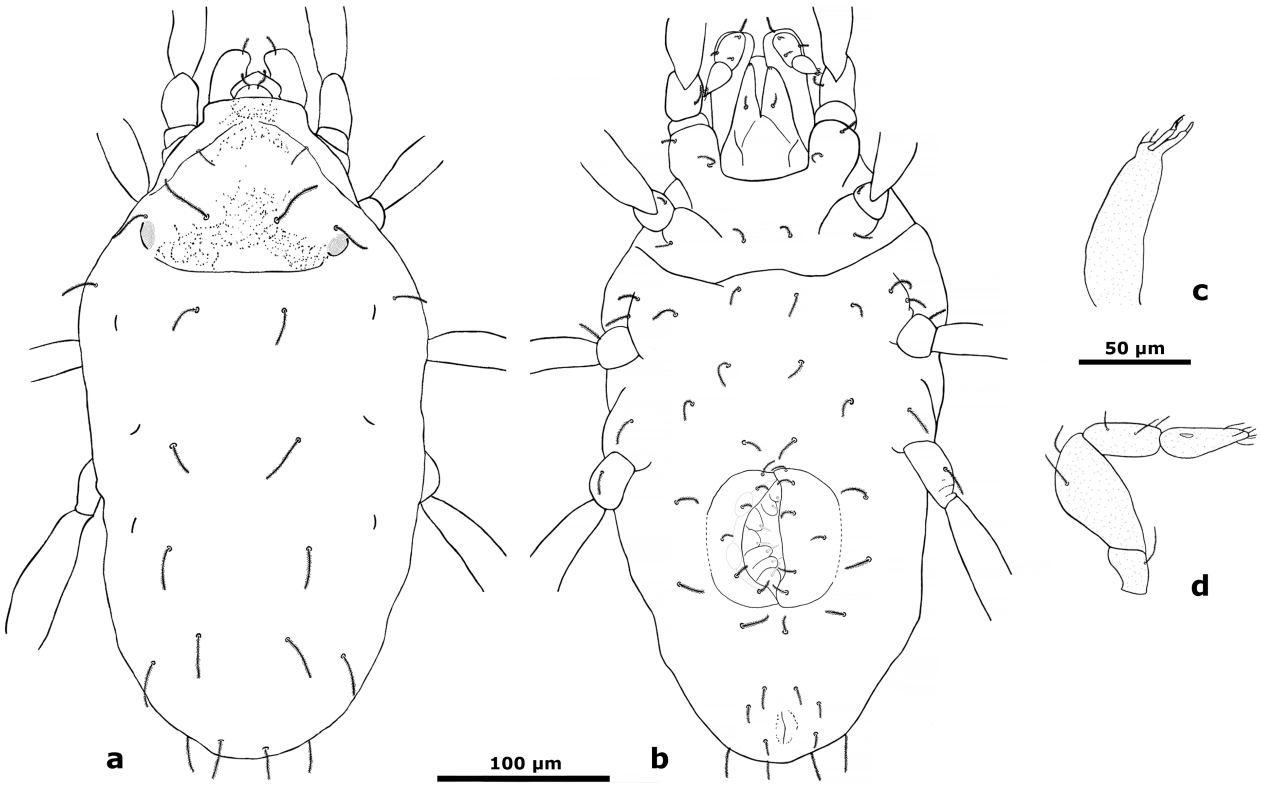

Figure 4. *Stereotydeus ineffabilis*. (**a**) Dorsal view; (**b**) ventral view; (**c**) chelicera; (**d**) lateral view of the pedipalp.

Ventral side: As in the other species of the genus, the genitalia are situated in a circular camerostome protected by two flaps which, under the optical microscope, laterally are hardly separated from the body wall (as in *S. mollis*) (Figures 4b and 8b). Seven pairs of internal genital setae (Figures 4b and 8b). Each genital cover has 6 setae of which the fourth is more lateral. The aggenital setae are present in 5 pairs, but often the specimens observed showed asymmetry in the number with a total of 9 setae (Figures 4b and 8b). The anal pore in ventral and distal position, is smaller than the genital pore and it is covered by two flaps and surrounded by 3 pairs of plumose setae.

Gnathosoma: Rostrum triangular with 2 pairs of nude apical setae. Chelicerae plump, dotted; movable arm about the same length of the fixed arm; two setae at the base of the

movable digit and shorter of the digit itself (Figure 4c). Pedipalps dotted with the terminal segment slender and same length as the subterminal segment (Figure 4d) with basal dorso-lateral rhagidiform organ and 7 apical setae.

Legs: The legs are slender and shorter than the body: the second pair is shorter than the other pairs but almost comparable in length with the third while the first pair is slightly shorter than the fourth (Figures 6 and 7). Coxal setal formula: 3, 1, 4, 3. All femora are undivided. Solenidia: basal and dorsal on genua I, II, III; at 2/3 (mid-basal) of the tibia's length in all legs of the specimens. As for the *S. mollis,* tibiae I and II with a small apical rhagidial organ (Figures 8b and e). Tarsi I and II with three rhagidiform organs (the basal oblique and slightly longer than the two apical ones) in confluent fields (Figure 8b) but in Inexpressible Island samples with three rhagidiform organs on tarsi I and II with different sizes: the apical round and smaller than the other and not aligned in a continuous (or confluent) field (Figures 5 and 8d); a visible small nude round seta is present at the base of the latter rhagidial organ on tarsi I and II. Chaetotaxy of the legs very variable: trochanters with 1 seta, femura I, II, III and IV with 16 (or 15; one is lighter and less developed, sometimes missing) in PRI and 17 in INE, 15, 9 (PRI) or 11 (INE) and 8 setae respectively; genua I with 8 setae and II with 7 (PRI) or 6 (INE) and genua III and IV with 5 setae each; all tibiae with 6 setae each; tarsi I, II, III and IV with 18 (sometimes 17 in INE), 13, 12 (PRI) or 9 (INE) and 13 respectively; leaf shaped, brush-like setae between the claws on top of the empodia (Table 6; Figures 6 and 7).

Table 6. Chaetotaxy of *S. ineffabilis* legs. Dorsal and ventral refer to surfaces of the leg segments; solenidia are excluded from the count while trichobotria are included (see **Figures 6 and 7** for the details and position concerning the legs chaetotaxy).

Area		Leg	Coxa	Trochanter	Femur	Genu	Tibia	Tarsus
Prior Island	Dorsal	I	0	0	10-11	5	3	8
		II	0	0	9	4	3	5
		III	0	0	6	2	3	4
		IV	0	0	4	3	3	5
	Ventral	I	3	1	5	3	3	10
		II	1	1	6	3	3	8
		III	4	1	3	3	3	8
		IV	3	1	4	2	3	8
Inexpressible Island	Dorsal	I	0	0	10	5	3	7-8
		II	0	0	9	3	3	5
		III	0	0	7	2	3	4
		IV	0	0	4	3	3	5
	Ventral	I	3	1	7	3	3	10
		II	1	1	6	3	3	8
		III	4	1	4	3	3	5
		IV	3	1	4	2	3	8

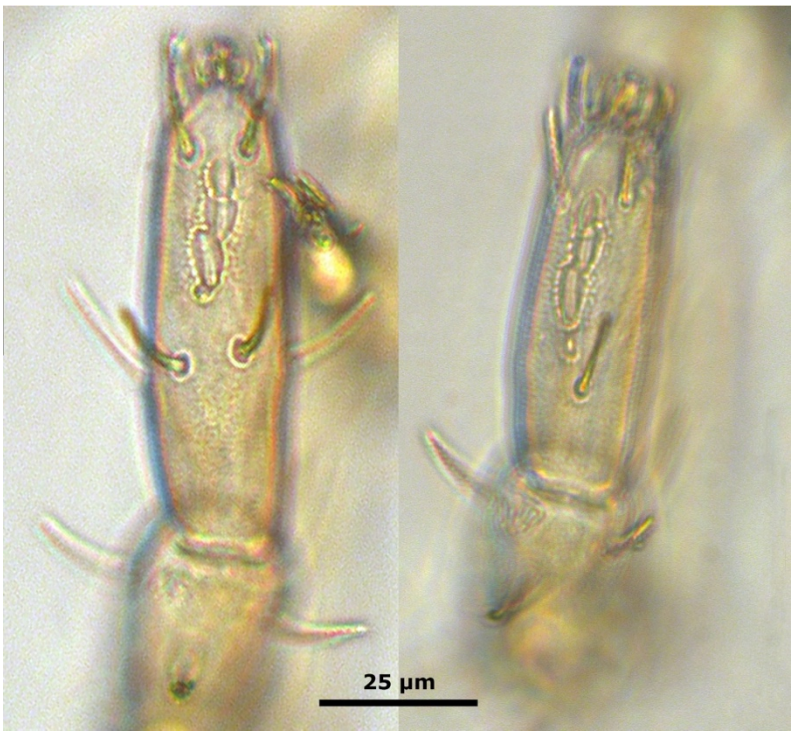

Figure 5. Asymmetry of the rhagidial organs on tarsi I (left) and II (right) in the specimens from Inexpressible Island. Apical rhagidial organs on tibiae I and II also visible.

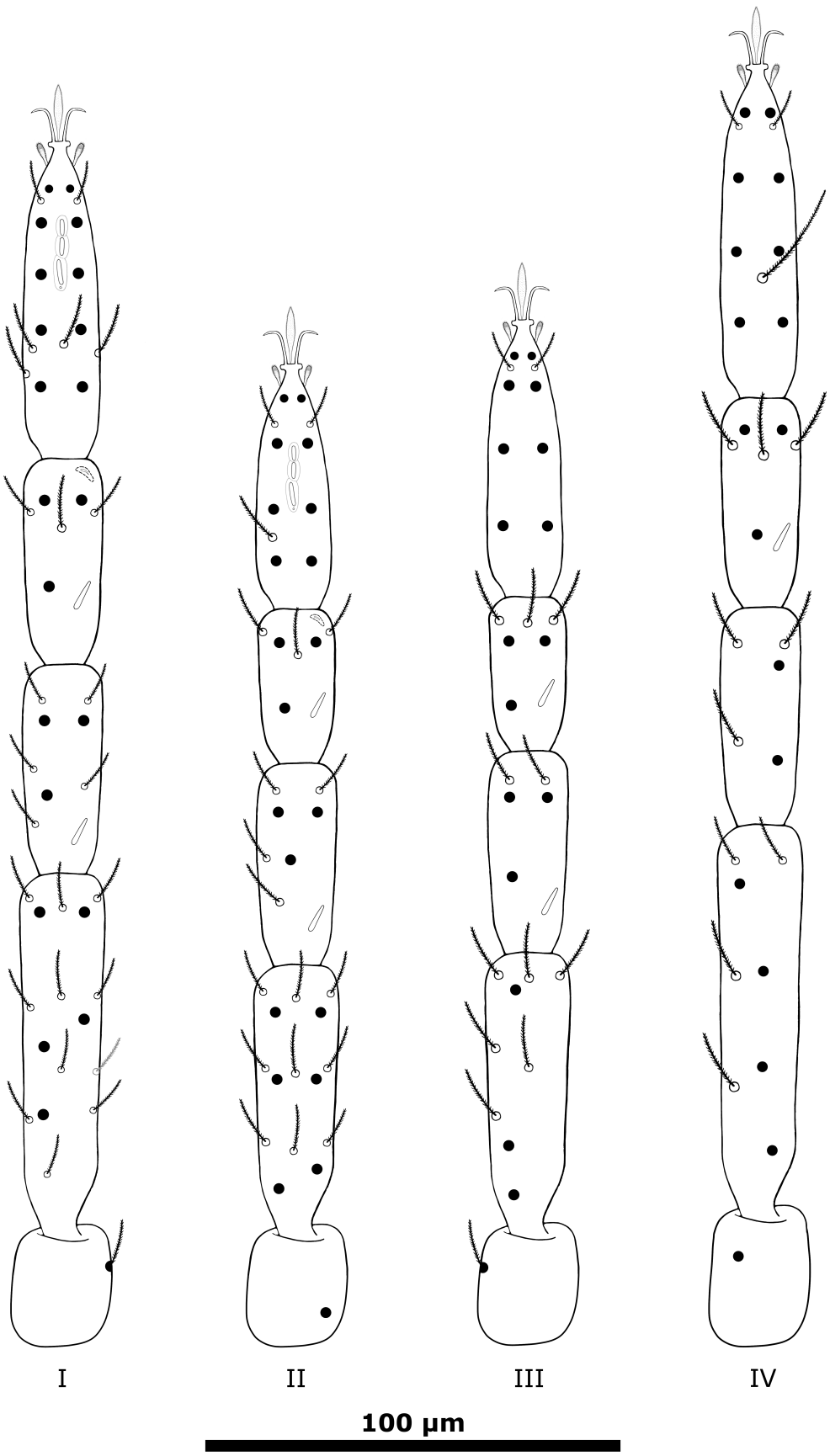

Figure 6. Chaetotaxy of *S. ineffabilis* legs of Prior Island specimens; black dots represent the setae on the ventral side; light grey indicates setae that are missing sometimes; outlines after the drawings of Pittard (1971) arranged with proportions and details of *S. ineffabilis* individuals from Prior Island.

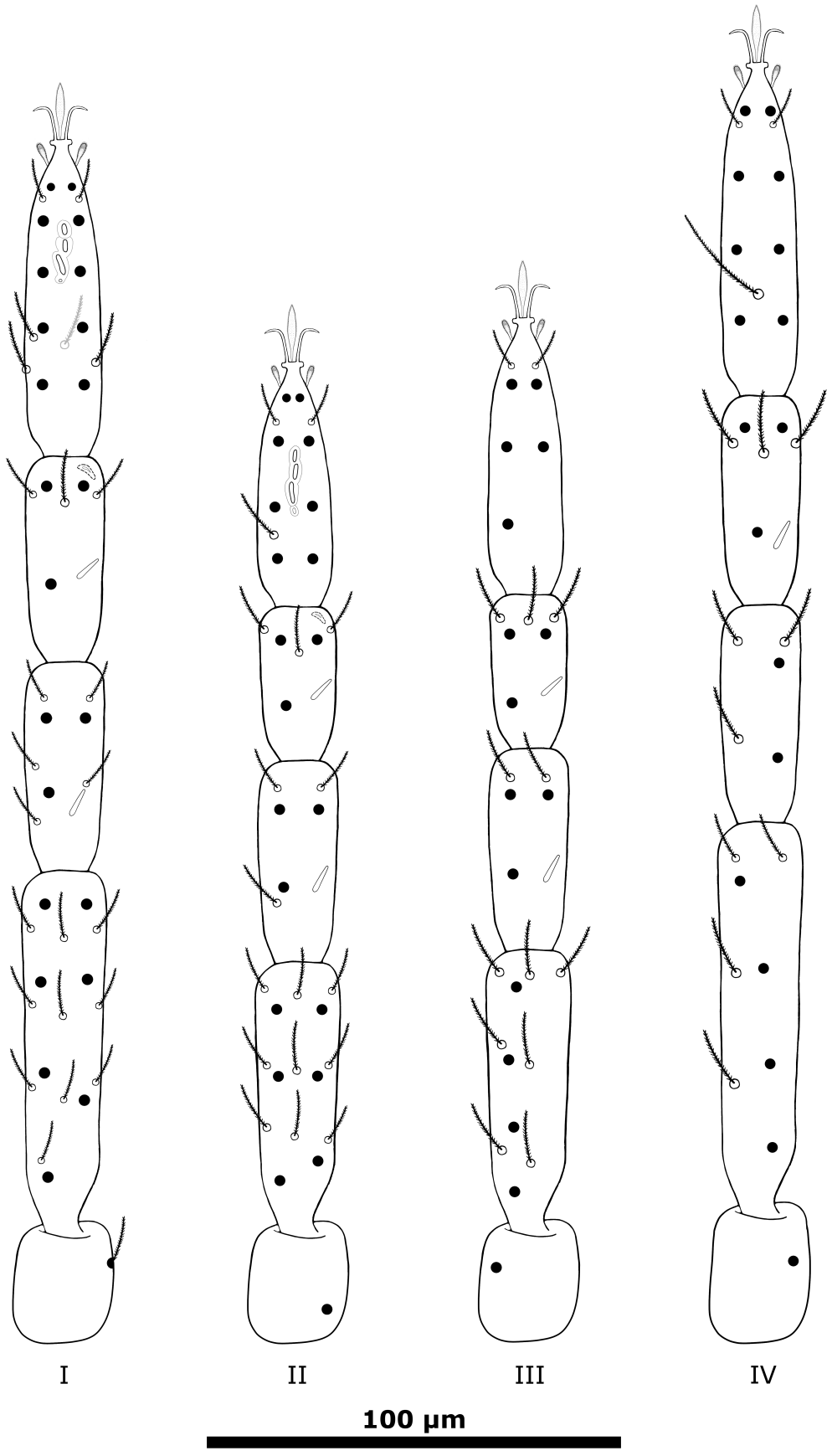

Figure 7. Chaetotaxy of *S. ineffabilis* legs of Inexpressible Island specimens; black dots represent the setae on the ventral side; light grey indicates setae that are missing sometimes; outlines after the drawings of Pittard (1971) arranged with proportions and details of *S. ineffabilis* individuals from Inexpressible Island.

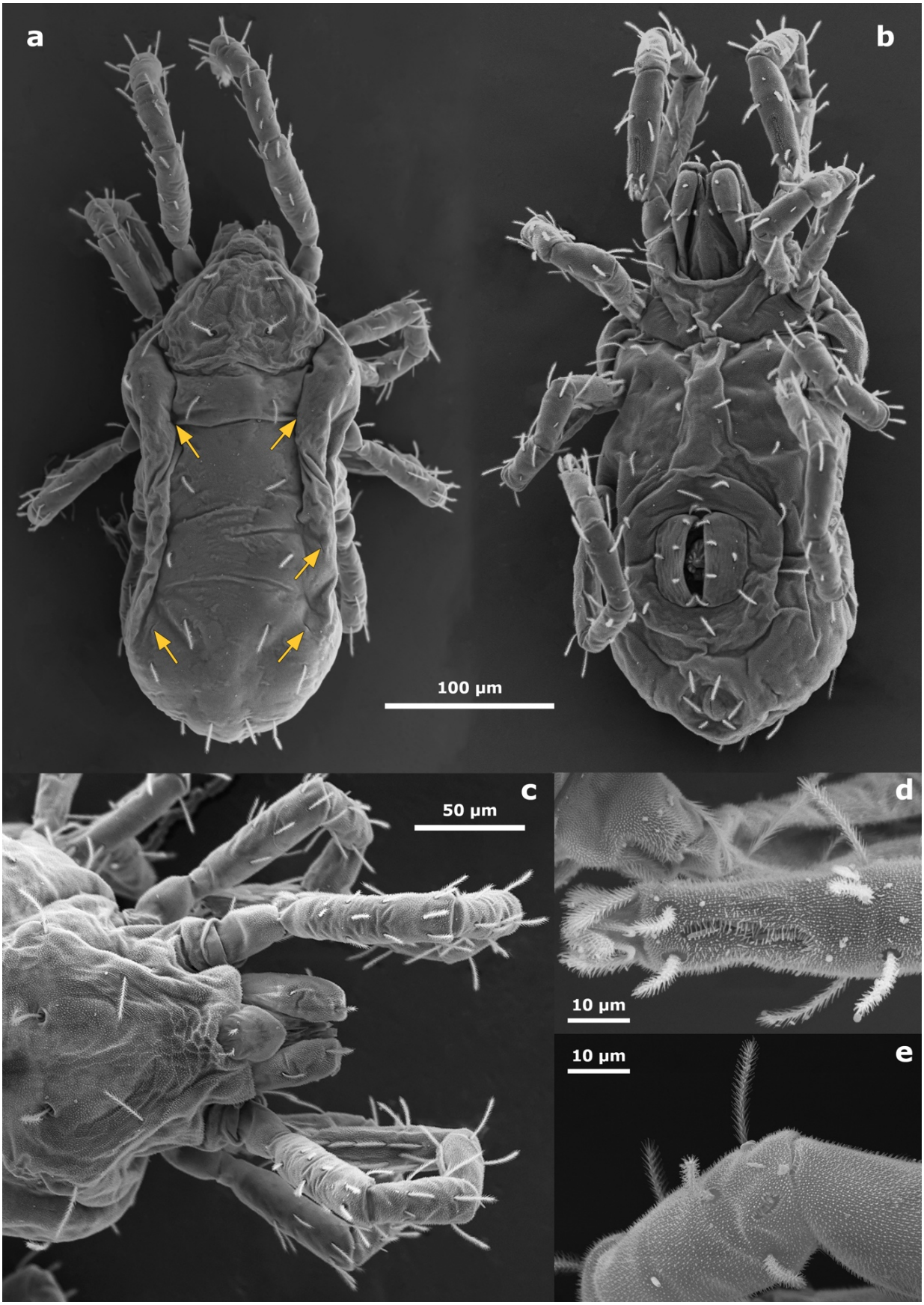

Figure 8. Scanning electron microscopy. *S. ineffabilis* (**a**) dorsal view, slit pores visible in correspondence of the orange arrows; (**b**) ventral view with internal genital setae and asymmetry in the aggenital setae visible; (**c**) detail of the propodosoma, epirostrum visible; (**d**) asymmetry in the rhagidial organs on tarsus I of a specimen from Inexpressible Island; (**e**) tibia I, rhagidial organ and solenidium visible.

Remarks: Comparing the *S. ineffabilis* adults with *S. mollis* as described in Womersley & Strandtmann (1963), Strandtmann (1967), Pittard (1971) and Pittard *et al.* (1971), the characters that help to positively identify and distinguish the adults of *S. ineffabilis* from *S. mollis* were: *i)* the smaller size of the adults of the former species than the latter; *ii)* the number of the aggenital setae (10 or 9, in *S. ineffabilis* while 8 in *S. mollis*); *iii)* the asymmetry in the tarsal rhagidial organs in *S. ineffabilis* specimens; *iv)* the terminal (4th) segment of the pedipalps as long as the subterminal (3rd) and bearing 7 setae in *S. ineffabilis* (while in *S. mollis* the 4th segment has a cluster of 8 setae and is longer than the 3rd segment); *v)* the equal length of the movable and fixed arms of the chelicerae in *S. ineffabilis* and *vi)* the position of the solenidia on the tibiae and the genua. Only two specimens (one male from Campo Icaro and one from Inexpressible Island) prepared for the preliminary morphological analyses and observed under the optical microscope, together with all the *S. ineffabilis* characters showed a wrinkle (like a hint of division) at the apical quarter of the femora (in I and II the wrinkle is barely visible while in III and IV is more clear) and the position of the anal pore ventral but almost terminal. Another specimen from INE prepared for the SEM shows wrinkles at the terminal part of the femora. It is possible then, that the sign of femora divisions observed with optical microscopy technique in the aforementioned species are just artifacts. Unfortunately, genetic data are not available for these specimens.

- ***Stereotydeus nunatakis*** sp. nov.: Brunetti.

Type Locality: 75°53'57"S 162°35'08"E, Starr Nunatak, South Victoria Land, Continental Antarctica.
Holotype: slide STN1, male, 11 January 2019. Deposited at the Collection of the Department of Life Sciences at the University of Siena.
Paratype: STN2, male, Starr Nunatak, 11 January 2019; STN3_P6, female, 75°41'31"S 162°52'34"E, Prior Island, South Victoria Land, Continental Antarctica, 11 January 2019; STN4_S6, female, Starr Nunatak, 11 January 2018. Deposited at the Collection of the Department of Life Sciences at the University of Siena.

Material Examined for the Description

Starr Nunatak, 1+6 slides (2+2♀, 0+2♂, 0+2 nymphs) and Prior Island, 2 slides with single individuals (2 ♀). The specimens will be conserved at the Collection of the Department of Life Sciences at the University of Siena.

Etymology: named after the ice-free ridge at the holotype locality, Starr Nunatak, continental Antarctica.

Description: Soft-bodied mite well sclerotized with a clearly visible sculptured pattern on the dorsum. The body length of the Holotype is 566,89 μm; the average length of the adult specimens studied is 563 μm, with values ranging from 537 to 582 μm (± 13,57). The adults of the species are comparable in size with *S. belli* (Womersley & Strandtmann 1963). The shape of the body is similar to the other species of the genus and the furrow dividing the propodosoma from the hysterosoma is evident both ventrally and dorsally.

Dorsal side: The epirostrum is strongly trilobed and lightly striated and the epivertex is almost smooth with two ciliated setae at the base of the middle lobe (Figures 9a and 11d). Propodosomal area is strongly sclerotized, more than the rest of the dorsum showing an evident reticulated pattern that lightly fades in the proximity of the sejugal furrow (Figures 9a and 11d). The eyes are convex and striated. Three pairs of slit pores are present on each

side of the dorsum: the first pair is horizontal (parallel to the sejugal furrow) and the two other pairs are oblique (Figure 9a); as for other species of the genus, the hysterosoma carries 8 pairs of plumose setae (as shown in the Figures 9a and 11a) almost all of the same length.

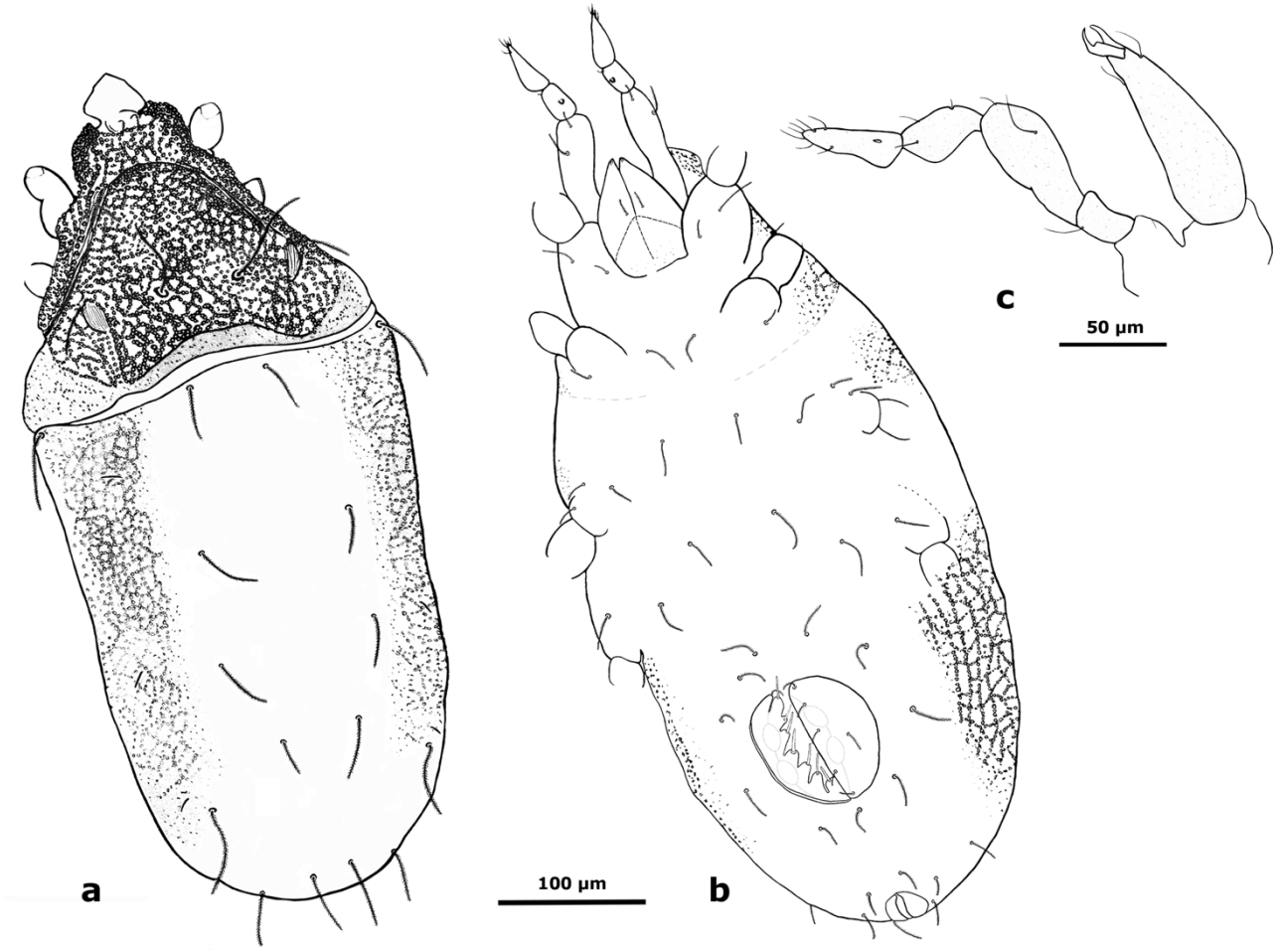

Figure 9. *Stereotydeus nunatakis*. (**a**) Dorsal view; (**b**) ventral view; (**c**) lateral view of chelicera and pedipalp.

Ventral side: As for the other species of the genus, the genitalia are situated in a circular camerostome protected by two flaps which laterally are well distinct from the body wall (as in *S. belli*). Seven pairs of internal genital setae (Figure 9b). Each genital cover holds 6 setae of which the fourth is more lateral. The aggenital setae are present in 5 pairs, but were also observed specimens with asymmetry in the number (4/5 and 5/6) (Figures 9b and 11b) The anal pore in ventral and distal position, is smaller than the genital pore and it is covered by two flaps and surrounded by 3 pairs of plumose setae.

Gnathosoma: Rostrum triangular with 2 pairs of apical nude setae. Chelicerae are plump, dotted and the movable arm is longer than the fixed arm (Figure 9c). Two nude setae, one is long as the movable digit at the base of the fixed arm while the second is about half of the length of the movable digit and it is positioned lower than the latter. Pedipalps dotted with the terminal segment slender and same length as the subterminal segment with 7 apical setae and basal dorso-lateral rhagidiform organ (Figures 9c and 11c).

Legs: The legs are slender and shorter than the body: the second pair is shorter than the others, the fourth pair is longer than the rest while the first and third pairs are almost comparable in length (Figure 10). Coxal setal formula: 3, 1, 4, 3. Trochanters all bearing 1 seta. All femora are divided. Solenidia: mid-basal (2/3) on tibiae I, II and IV and genua I and II, medial on tibia and genu III (but sometimes observed also mid-basal). Small apical rhagidial organ presents also on tibiae I and II. Tarsi I and II with three symmetrical rhagidiform organs comparable in length laying in a common field (Figure 11e); a visible small nude round seta is present at the base of the latter rhagidial organ on tarsi I and II. Chaetotaxy of the legs: femora: 12/5, 10/5, 7/4, 6/2; genua: 11, 6, 5, 5; all tibiae with 6 setae each; tarsi: 21, 14, 12, 15; Legs setae plumose except for 1-2 pairs at the tips of all tarsi clavate and brush-like (Figure 11f); bulb-shaped, brush-like setae between the claws on top of the empodia (Table 7; Figure 10).

Table 7. Chaetotaxy of *S. nunatakis* legs. Dorsal and ventral refer to surfaces of the leg segments; chaetotaxy in the femur shown as basal/apical segment; solenidia are excluded from the count while trichobotria are included (see **Figure 10** for the details and position concerning the legs chaetotaxy).

	Leg	Coxa	Trochanter	Femur (divided)	Genu	Tibia	Tarsus
Dorsal	I	0	0	9/3	4	3	9
	II	0	0	6/3	3	3	6
	III	0	0	5/3	2	3	4
	IV	0	0	2/2	3	3	7
Ventral	I	3	1	3/2	7	3	12
	II	1	1	4/2	3	3	8
	III	4	1	2/1	3	3	8
	IV	3	1	4/-	2	3	8

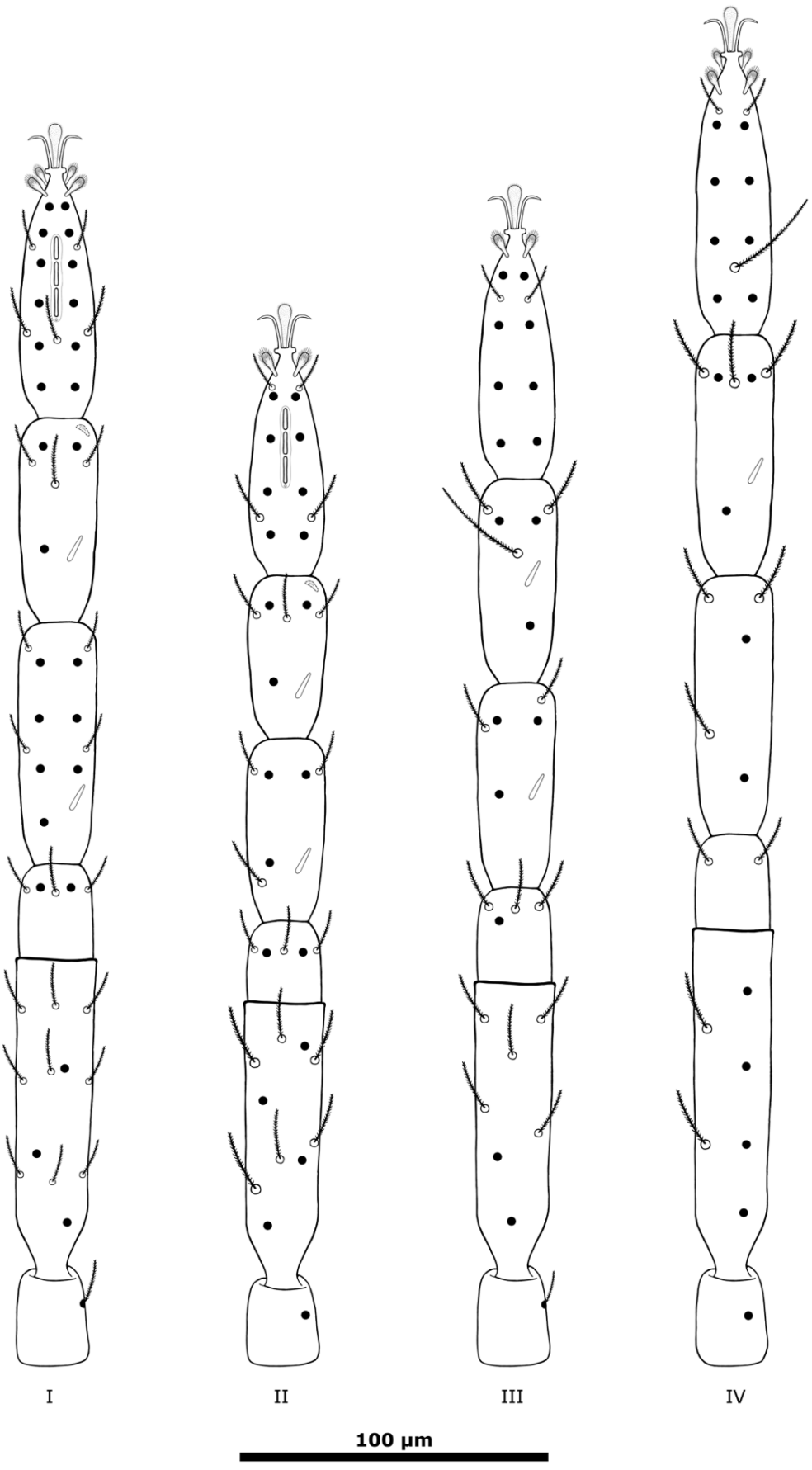

Figure 10. Chaetotaxy of *S. nunatakis* legs; black dots represent the setae on the ventral side; outlines after the drawings of Pittard (1971) and arranged with proportions and details of *S. nunatakis* individuals.

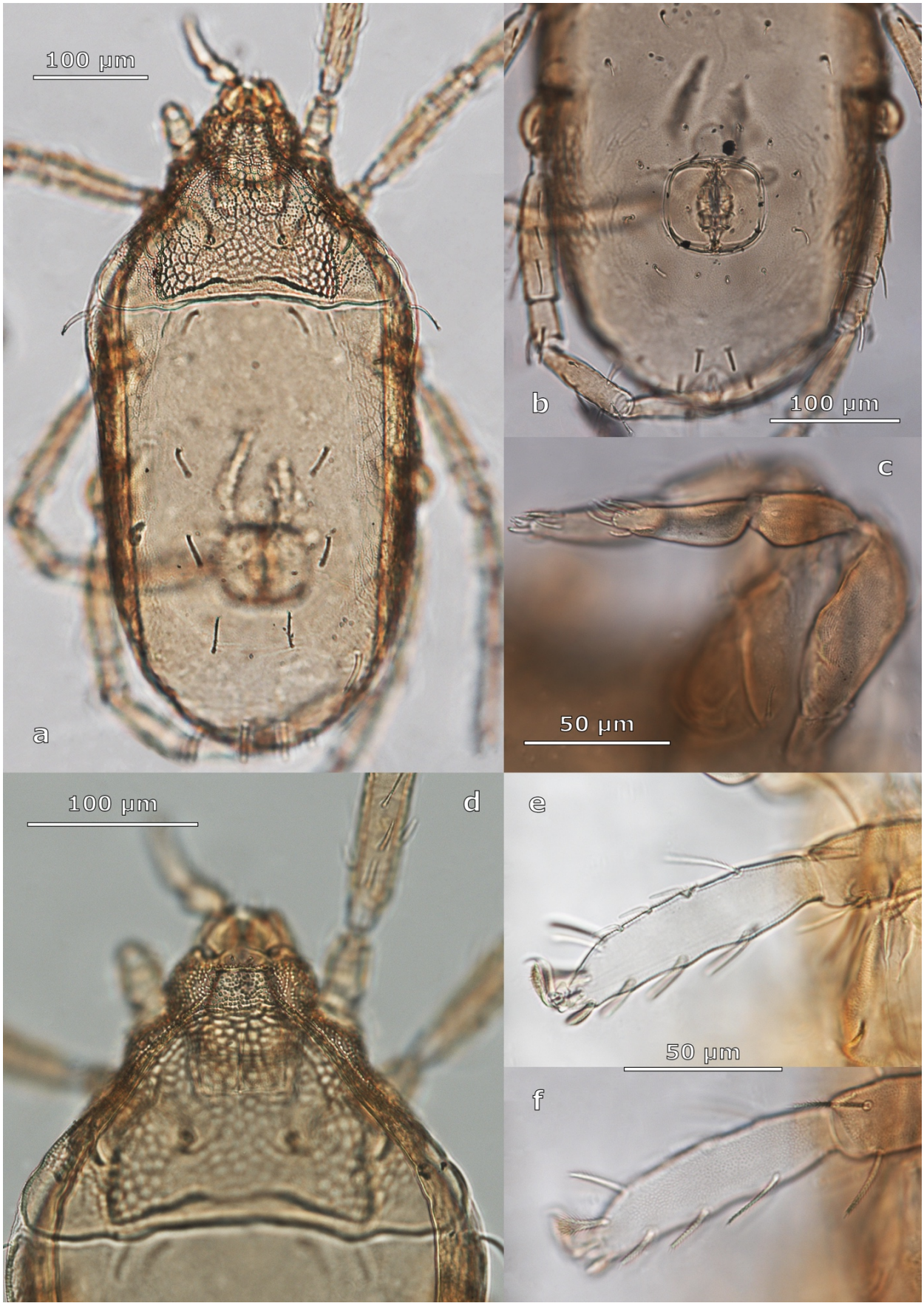

Figure 11. *S. nunatakis* (**a**) dorsal view; (**b**) ventral view with evident circular camerostome and visible asymmetry of the aggenital setae; (**c**) detail of the pedipalps (**d**) detail of the propodosoma; (**e**) lateral view of the rhagidial organs on tarsus II; (**f**) clavate setae on top of tarsus II.

Remarks: Comparing the *S. nunatakis* adults with the other *Stereotydeus* species, appeared to be more closely related to *S. punctatus* as described in Strandtmann (1967) and to *S. belli* as described in Womersley & Strandtmann (1963) and Strandtmann (1967) for the division of all the femora at the apical quarter, the prominent dorsal sculpturing and the apical (4th) segment of the pedipalps as long as the third while for the size of the adults, the number and position of the rhagidial organs on tarsi I and II the specimens of *S. nunatakis* are comparable only with *S. belli* as described in Womersley & Strandtmann (1963). Conversely, the characters that help to positively identify and distinguish the adults of *S. nunatakis* from both *S. belli* and *S. punctatus* were: *i)* the number of the aggenital setae (10 with possible asymmetry at 9 or 11 in *S. nunatakis* while 8 in *S. punctatus* and 22 in *S. belli*); *ii)* the terminal (almost apical) position of the anal pore in *S. nunatakis* (while subterminal in *S. belli* and dorsal in *S. punctatus*); *iii)* the position of the solenidia is mid-basal on tibiae and genua I,II and tibia IV and medially on tibia and genu III in *S. nunatakis* (while in *S. punctatus* are all basal on tibiae I-IV and genua I-III, and in *S. belli* are apical on tibiae and genua I, II and tibia III and medial on genua III and tibia IV); *iv)* the bulb-shaped brush-like setae on top of the tarsi in *S. nunatakis* (while in *S. punctatus* and *S. belli* the setae are slender and plumose, not different from all the others present on the legs); *v)* the 4th segment of the pedipalps bearing 7 plumose setae in *S. nunatakis* (while are 8 in *S. belli*) and finally *vi)* the chaetotaxy of the legs.