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Edward A Ueckermann, Raf M J de Vis, Eva Reybroeck, Lore Vervaeet, Wendy van Lommel, et al.. Redescription of *Pronematus ubiquitous* (McGregor, 1932) (Acari, Iolinidae), description of two new species and redescription of two additional species with a review of and key to all *Pronematus* species. *Acarologia*, 2024, 64 (1), pp.277-311. 10.24349/tyki-9xlp . hal-04495712

HAL Id: hal-04495712

<https://hal.science/hal-04495712>

Submitted on 8 Mar 2024

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Redescription of *Pronematus ubiquitous* (McGregor, 1932) (Acari, Iolinidae), description of two new species and redescription of two additional species with a review of and key to all *Pronematus* species

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Original research

ABSTRACT

Pronematus ubiquitous (McGregor) is redescribed based on specimens collected at the type locality. Additionally, in a survey for natural enemies of *Aculops lycopersici* (Tryon), *P. ubiquitous* was found in most European countries where samples were gathered as well as in Morocco. Together with specimens available from museum collections and originating from Brazil, South Africa and Türkiye, the newly collected specimens were measured and morphologically compared with those from the type locality. Additionally, single female expansion lines were set-up with specimens from California (USA), Belgium, France, Italy, The Netherlands and Morocco, and molecular analysis were performed in order to construct a phylogenetic tree. The tree showed differences between origins, but this was assigned to intraspecific variation. A PCA analysis on morphological characters separated the Brazilian specimens from those of all other geographical origins. The Californian (USA) specimens grouped together, as did the Moroccan and all European specimens, but with overlap between the groups. The Brazilian specimens were described as a new species, *P. brasiliensis* n. sp. All other specimens were considered as *P. ubiquitous*. Furthermore, we searched for types of early described *Pronematus* species and studied *P. rykei*, *P. karrooi* and *P. tenuisetosus* types; the first two species were confirmed morphologically as a distinct *Pronematus* species while the latter belongs to the genus *Pseudopronematus*. The morphological characteristics of specimens that were used for a recent redescription of *P. rykei* did not coincide with our redescription of the type. We transferred those specimens to the new species, *P. juglandi* sp. nov.

Received 19 October 2023

Accepted 07 February 2024

Published 29 February 2024

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Academic editor

Faraji, Farid

<https://doi.org/10.24349/tyki-9xlp>

ISSN 0044-586X (print)

ISSN 2107-7207 (electronic)



Ueckermann E. A. & De Vis R. M. J. *et al.*

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Keywords mites; Pronematinae; Iolinidae

Zoobank <http://zoobank.org/A944B2C0-C415-4747-974F-7334BAB826E8>

How to cite this article Ueckermann E. A. & De Vis R. M. J. *et al.* (2024), Redescription of *Pronematus ubiquitous* (McGregor, 1932) (Acari, Iolinidae), description of two new species and redescription of two additional species with a review of and key to all *Pronematus* species. *Acarologia* 64(1): 277-311. <https://doi.org/10.24349/tyki-9xlp>

Introduction

Canestrini (1886) described the genus *Pronematus* based on the type species *P. bonatii* Canestrini, 1986 (André 2021). Baker (1965) introduced the leg setal pattern in the genus description and André (1980) made additional changes to that description, adding the two minute and mostly overlooked unguinal setae of tarsus I (totalling 8 instead of 6 on tarsus I).

McGregor (1932) described *Tydeus ubiquitous* based on specimens collected from citrus in Lindsay, California (USA). Thor (1933) redescribed the species and assigned it to the genus *Pronematus*. Since the first description of *P. ubiquitous*, 8 redescrptions have been made (Thor 1933; Meyer and Ryke 1959; Ryke and Meyer 1960; Meyer and Rodrigues 1966; Baker 1946; Baker 1965; Baker 1968; André 1980). All these redescrptions were minimal and none of them made measurements of relevant characters. This makes separating species within the genus uncertain, leaving space for interpretation. A thorough species description of *P. ubiquitous* can avoid misidentifications or the description of new species that in fact are not. According to Ueckermann and Grout (2007), “the condition of the type material of *P. ubiquitous* renders an examination impossible (Dr. Ron Ochoa, personal communication)”. This sets out the first objective of this paper: redescrbing *P. ubiquitous* based on new material from the type locality.

Since 2016, *Aculops lycopersici* (Tryon, 1917) (Acari: Eriophyidae) has become a major pest in greenhouse tomato crops in Western Europe (Reybroeck *et al.* 2018; Vervae *et al.* 2021). Therefore, a search for potential natural enemies was started in 2017 in Belgium and neighbouring countries during two successive Flemish projects (DUCATO and BALTO) (Reybroeck *et al.* 2018; Vervae *et al.* 2021; Vervae *et al.* 2022). Special attention was given to members of the subfamily Pronematinae of the family Iolinidae because: i) literature suggested that members of this subfamily are potential biocontrol agents against eriophyid mites (Abou-Awad 1979; Abou-Awad *et al.* 1999; Knop and Hoy 1983a; Knop and Hoy 1983b; Vervae *et al.* 2021); ii) Pronematinae are reported in Europe (De Jong *et al.* (2014), Fauna Europaea, accessed on 13/2/2023) although not very often; iii) despite a plethora of efforts by the biocontrol community, not a single phytoseiid mite (mostly used in biocontrol programs) was found to be able to establish successfully on tomato (Drukker *et al.* 1997; Castagnoli *et al.* 1999; Cédola *et al.* 2001; Van Houten *et al.* 2013; Paspatis *et al.* 2021); and iv) following our early findings with Pronematinae (Vervae *et al.* 2021; Vervae *et al.* 2022), biocontrol companies showed a renewed interest to explore the potential of these species (van Houten *et al.* 2020; Pijnakker *et al.* 2022a). In our studies, we focused on *P. ubiquitous* as a candidate for the biological control of the tomato russet mite in greenhouse tomato (Aussems *et al.* 2021; Vervae *et al.* 2021; Vervae *et al.* 2022). Its importance as a biological control agent might also increase as it can suppress not only *A. lycopersici* but also powdery mildew (Pijnakker *et al.* 2022b). The species has recently been registered as biological control agent in several European countries (D. Vangansbeke, personal communication) and the species was added to the so-called “Positive List” of EPPO (PM 6/3(5), https://www.eppo.int/RESOURCES/eppo_standards/pm6_biocontrol). As we found morphological variability between specimens from different origins, we set out to confirm the identity by comparison not only morphologically but also genetically with material from the type locality. At present, DNA sequences are used in the delineation and identification of species. Several studies show that sequence diversity in a near 650 bp region near the 5' region of the COI gene provides strong species level resolution for different animal groups including insects (Hebert *et al.* 2003; Hebert *et al.* 2004; Boehme *et al.* 2012; Ovalle *et al.* 2014) and mites (Li *et al.* 2012; Doña *et al.* 2015; Mağol *et al.* 2019; Schäffer *et al.* 2019). Other authors used other genes with success (Tixier *et al.* 2008; Okassa *et al.* 2010; Queiroz *et al.* 2021) although with difficulties for some mite species (Tixier *et al.* 2017). An essential requirement for the DNA barcoding methodology is the linkage of species identification to a voucher specimen within a curated biological collection (Puillandre *et al.* 2012). This association facilitates later investigations and establishes a framework for validating the accuracy of species identification. Establishing this link in the Pronematinae comprises the second objective of our study.

Third, we compared specimens from museum collections from other regions and countries i.e., South Africa, Brazil, and Türkiye with the new type material. *P. ubiquitous* is considered cosmopolitan (Meyer and Rodrigues 1966) with records in South America (De Vis *et al.* 2006b; Fiaboe *et al.* 2007; Sousa *et al.* 2015), North America including Hawaii (McGregor 1932; Denmark and Porter 1973; Goff 1987; Acuña-Soto *et al.* 2017), Asia (Gerson 1968; Gupta 2002; Baradaran and Arbabi 2009; Barbar 2016; Darbemamieh *et al.* 2021), Europe (Carmona 1970; Castagnoli 1984; Karg 1991; Kumral and Çobanoğlu 2015; Vela *et al.* 2017; van Houten *et al.* 2020), Africa (Meyer and Rodrigues 1966; Abou-Awad *et al.* 1999; Ueckermann and Grout 2007) and Oceania (Maynard *et al.* 2018). However, as no measurements are available, we wanted to check at least part of these identifications and confirm at least partially the 'ubiquitous' distribution of *P. ubiquitous*.

Finally, we reviewed the genus *Pronematus* taxonomically. Baker (1968) made a first review and recognised 13 species of which Kaźmierski (1998) retained 3 species (*P. ubiquitous*, *P. rykei* Meyer & Rodrigues, 1966 and *P. sextoni* Baker, 1968). More recently, André (2021), (<https://species.wikimedia.org/wiki/Pronematinae>, accessed on 12/2/2023), made a new overview recognising 11 species (*P. bengalensis* Gupta & Paul 1985; *P. debilis* Tseng, 1985 (erroneously mentioned as *P. dibilis* on the website above); *P. indiana* Gupta & Paul 1985; *P. karrooi* Ryke & Meyer, 1960; *P. oryzae* Menon, Joshi, Kumar & Ramamurthy, 2007; *P. perpusillus* Tseng, 1985; *P. rykei* Meyer & Rodrigues, 1966; *P. saularis* Gupta & Paul, 1992; *P. sensillaris* Ryke & Meyer, 1960; *P. sextoni*; and *P. ubiquitous*), and retained a list of 12 species *inquirendae* (including *P. tenuisetosus*). The descriptions of the species *inquirendae* lack details on the number of setae on the leg segments, measurements of the setae, or drawings of legs and ventral part which are required to be assigned to a genus. It appears that the Baker species will remain in that state forever, as the types have faded away, are deteriorated, or lost (personal observation of the first author). We studied several species that are held in South African museum collections, in order to add new information to their original descriptions and constructed a key for the confirmed species.

Material and methods

Collecting material

Pronematus ubiquitous specimens were collected near the type locality (Lindsay, California (USA)) in 2019, mounted and sent to the first author in 2021. To perform genomic studies, a second collection near the type locality was done during the last trimester of 2021. Part of the specimens were mounted on slides and others were used to start a laboratory population according the methods of Vervaeke *et al.* (2022). From this population, new subpopulations starting from one single female (single female expansion lines, SFEL) were set up. These SFEL are necessary to obtain sufficient genetically homogeneous material coming from one female because it is difficult to extract DNA successfully from one mite. Ideally, a minimum of 4 females is needed. By the end of 2021, of each SFEL some specimens were used to make slides and the rest of the population was transferred to separate vials with 99% alcohol. All material was sent to Ghent University for molecular analysis, morphological studies were done at PSKW (Research Station for Vegetable Production).

During the DUCATO and BALTO projects (Reybroeck *et al.* 2018; Aussems *et al.* 2021), a survey was done from summer 2017 until autumn 2019, mostly in Belgium and France (Survey permission nr. NOR TREL1902817S/165). Sampling was directed to wild or cultivated eriophyid susceptible plants (mostly *Rubus* spp., but also *Vitis vinifera*, *Ficus carica*, *Tilia*, *Acer*, etc.) and wild or cultivated solanaceous plants (*S. dulcamara*, *Brugmansia* spp., tomato, sweet pepper, egg plant, *Physalis* sp.). Sampling was done in the surroundings of the research institutes or during travel in Belgium or abroad. Cultivated solanaceous plants were sampled on organic farms in Flanders or in gardens and for wild plants on sites where they were earlier found according to the site waarneming.be.

In order to assure that the leaves were colonised by mites, about 20 fully developed leaves were picked per plant species per site. These leaves were placed in plastic bags, which were then closed assuring sufficient air was included to avoid desiccation, compression or damaging of the leaves. In most cases, the geolocation of the collection site was recorded. Samples were transported to PSKW where they were kept cool until processing. The samples were screened for predatory mites under a stereo microscope (Leica DM16 or Leica M125 C) and slide mountings were made of phytoseiids, iolinids and triophtydeids. All mounts were done using Hoyer medium (Krantz and Walter 2009). At the same time, small lab colonies were set up with iolinids and transferred to Ghent University where cultures were increased and SFEL were set up as to confirm the identity and at the same time provide material for the genetic study.

Additionally, we received from Biobest slides or laboratory populations set up with samples of The Netherlands, UK, Italy, Spain, and Morocco.

Finally, slides from museum collections with specimens from Brazil, South Africa and Türkiye were included in the morphological study.

Taxonomic studies

Specimens were measured using either a Zeiss Axioskop TM Research microscope with a Zen Soft Imaging System with measuring tools or a Leica DM750 with an eyepiece micrometer or Leica Flexacam c3 camera and Leica LAS-X software. Line drawings were either made from photographs taken with the Zeiss system and prepared with a Wacom One 13" Pen display and edited using Adobe Illustrator CS5, or with a Leica DM750 with a phototube. DIC-photos were made with an Olympus BX51.

The nomenclature of André (1981a & b) is used, for setal nomenclature that of Kaźmierski (1989). Measurements are in μm and placed directly after the character.

Comparison of different origins and principal component (PCA) analysis

The measurements of reared (mass rearing or SFEL) females fell within the range of the wild specimens for Belgium, France, California (USA), or the other way around for countries where few wild specimens were available (The Netherlands, Morocco). So, for the PCA analysis the specimens of each country or rearings thereof were pooled. A total of 114 measured specimens were included: California (USA): 20; Belgium: 20, France: 20, The Netherlands: 10, Spain: 3, Italy: 10, Türkiye: 2, Morocco: 11, South Africa: 5, and Brazil: 13. The PCA analysis was done with PAST (version 4.03). Based on morphological measurements, convex hulls were created.

Differences in temperature, host plant or diet influence the morphometric characteristics of mites such as Phytoseiidae (Vangansbeke *et al.* 2015; Lopes *et al.* 2018; Tixier *et al.* 2021; De Melo Ferreira *et al.* 2021). Seeman and Nahrung (2018) showed that the body size of free-living mesostigmatid mites is in line with the Bergmann size cline (i.e., smaller species in warmer climates and vice versa). Here we assessed whether a similar trend was present in the sampled *P. ubiquitus* specimens. For this purpose, we correlated the average length of seta *eI* with the latitude of the collection location (centre of the country or region) of the respective species. Seta *eI* was used as this characteristic showed to have the highest loading in the first axis of the PCA analysis.

Molecular analysis and construction of a phylogenetic tree

DNA extraction. The first step of the DNA extraction procedure was different for living mites compared to mites preserved in ethanol (90%). When still alive, 1-4 mites (preferably females) from one SFEL were directly transferred to a 1.5 ml Eppendorf tube. Mites preserved in ethanol were first transferred to Whatman filter paper and incubated at 55 °C for 10 minutes until all absorbed ethanol had evaporated. Next, the mites were crushed and homogenized

with an Eppendorf micropestle in 20 μ L extraction buffer (100 mM NaCl, 10 mM Tris-HCl, 1 mM EDTA, pH 8.0) supplemented with 2 μ L proteinase K (10mg/ml). The Eppendorf tubes were incubated at 60 °C for 30 minutes and subsequently, proteinase K activity was stopped by heating at 95 °C for 5min. The homogenate was stored at -20 °C until used as DNA-template for PCR-reactions.

Barcoding

Molecular identification of the mites was done by sequencing a fragment of the cytochrome c oxidase subunit I gene (COI, mtDNA). The “universal” invertebrate DNA primers LCO1490: 5' GGT CAA CAA ATC ATA AAG ATA TTG G 3' and HCO2198: 5' TAA ACT TCA GGG TGA CCA AAA AAT CA 3' (Folmer *et al.*, 1994) were used for PCR amplification of a 710-bp fragment of the COI marker gene. PCR amplifications were performed using the GoTaq® G2 Flexi DNA Polymerase kit (Promega). The 50 μ L reaction mixtures contained 4 μ L DNA, 26.75 μ L nuclease-free water, 10 μ L 5X Colorless GoTaq Flexi Buffer, 3 μ L MgCl₂ (25 mM), 1 μ L dNTPs (25 mM each), 2.5 μ L of each primer (10 μ M) and 0.25 μ L GoTaq G2 Flexi DNA polymerase (5u/ μ L). A touchdown PCR approach was chosen to increase PCR sensitivity, specificity and yield (Korbie and Mattick 2008). The thermal conditions of the PCR are presented in Table 1. All PCR products were purified using the E.Z.N.A. Cycle Pure Kit (Omega Bio-tek Inc, Georgia, USA) following the manufacturer’s instructions. Electrophoresis was carried out in a 2% agarose gel in 0.5X TAE buffer for 30 min at 100 V. PCR products were sent for sequencing to LGC Genomics GmbH, Berlin, Germany. The obtained forward and reverse sequences were examined, and a consensus sequence was made using the BioEdit Software version 7.2.5.

Phylogenetic tree

The consensus sequences were aligned online using MAFFT version 7. In the alignment of the COIs of *P. ubiquestus* we also included COI sequences of a closely related species, *Homeopronematus anconai* (Baker, 1943) (samples and COI sequences subject of another paper in preparation), and a set of gradually less related prostigmatid mites (from previous studies, available at GenBank NCBI) i.e. *Microtydeus* sp. (MG317470.1), *Tydeus* sp. (MN360403.1), *Panonychus ulmi* (Koch, 1836) (NC_012571.1), *P. citri* (McGregor, 1916) (NC_014347.1), *Tetranychus urticae* Koch, 1836 (MN348791.1, KY922441.1) and *T. urticae*-London_mt_genome (Sterck *et al.* 2012), and the mesostigmatid mite *Varroa destructor* Anderson & Trueman, 2000 (AP019523.1) as outgroup species. A phylogenetic tree was constructed using the software W-IQ-TREE with 1,000 ultrafast bootstrap combined with automatic model finding through ModelFinder (Trifinopoulos *et al.* 2016; Kalyaanamoorthy *et al.* 2017; Hoang *et al.* 2018). According to the Bayesian Information Criterion, the best-fit model was determined to be K3Pu+F+G4, based on the likelihood scores for 88 different models. The resulting Maximum-likelihood consensus tree was rooted with the sequence of *V. destructor* and edited with iTOL version 5 (Letunic and Bork 2021).

Table 1 Thermal conditions of the PCR amplification of the COI gene.

Phase	Step	Temperature (°C)	Time (min)	ΔT	Cycles
Start	Initialization	95	03:00		
Phase 1	Denaturation	95	01:00		
	Annealing	50	01:00	-1	10
	Extension	72	01:30		
Phase 2	Denaturation	95	01:00		
	Annealing	40	01:00		35
	Extension	72	01:30		
Termination	Final Elongation	72	07:00		
	Final hold	25	∞		

ΔT = temperature difference in each cycle.

Deposits of type material

Pronematus ubiquitus: topotype and other specimens from Californian (USA) were deposited at USNM, Smithsonian Institution, Washington, D.C, USA. Specimens from California (USA), Belgium, France, The Netherlands, Morocco, Italy, including voucher specimens of COI sequences (i.e., slide mountings of specimens originating from the same SFEL as the respective COI sequence) are deposited at CBGP, INRAE, Montpellier, France. Slide number of the voucher specimens at CBGP can be found in table 4.

Pronematus brasiliensis n. sp., *P. rykei* and *P. karrooi*: see below.

Deposit GenBank. For every collection location, the *Pronematus ubiquitus* COI-sequences herein obtained, have been deposited in the Genbank database (SUB13342766) under their accession numbers (Table 4).

Results

Collecting material

Near the type locality (Lindsay, California, USA), samples were collected at 4 sites during the first collection and at 5 sites during the second collection.

In Europe, a total of 338 samples were collected mainly in Belgium (271) and France (50) (Survey permission UGent nr. NOR TREL1902817S/165) but also some in The Netherlands (2), Germany (3), Czech Republic (3), Switzerland (3), Austria(1), UK (3), Spain (1), Italy (1) and Morocco (1). Samples from the five latter countries were provided by Biobest without reporting on the total number of samples taken in those countries. From these, 11 samples from 8 localities in Belgium, 6 samples from 4 localities in France, 2 samples from 2 localities in The Netherlands and 1 sample from 1 locality for Spain, Italy, and Morocco, contained *P. ubiquitus*. All slide mounts that were studied morphologically are mentioned below.

Material used in morphological studies

Pronematus ubiquitus: **Belgium**: 27/9/2018, Machelen aan de Leie, 50.945049°N, 3.487808°E, cucumber, 2 females and 3 females from SFEL, 35.1, Justine Dewitte; 27/09/2018, Ternat, 50.866798°N, 4.159654°E, *Brugmansia* sp., 3 females from SFEL, 39.1 Raf De Vis; 27/09/2018, Ternat, 50.866798°N, 4.159654°E, *Brugmansia* sp., 4 females, 40.2.1, 40.2.3, 40.5.1, 40.5.4, Raf De Vis; 17/10/2018, Sint-Truiden, 50.807778°N, 5.133289°E, *Vitis vinifera*, 1 female, 66.1, Eva Reybroeck; 26/8/2019, Ruddervoorde, 51.089279°N, 3.171159°E, *Vitis vinifera*, 1 female, 123.1, Robin Van Haevermaet; 19/5/2020; Ruddervoorde, 51.089279°N, 3.171159°E, *Physalis* sp., 1 female, 154.1, Robin Van Haevermaet; 23/6/2019, Erembodegem-Aalst, 50.914291°N, 4.080081°E, *Brugmansia* sp., 1 female, 170.1-2, Raf De Vis; **France**: 29/09/2018, Treignat (La Villaageai), 46.362778°N, 2.349444°E, tomato, 1 female, 46.1, Dieter Foqué; 29/09/2018, Treignat (La Villaageai), 46.362778°N, 2.349444°E, sweet pepper, 1 female, 50.1 + 3 female from SFEL, Dieter Foqué; 29/09/2018, Treignat (La Villaageai), 46.362778°N, 2.349444°E, *Rubus idaeus*, 1 female, 55.2, Dieter Foqué; 8/2018, Hyères, 43.1124°N, 6.1638°E, Fig, *Ficus carica*, 1 female, 79.3, Lore Vervaet; 8/2018, Allan, 44.5120°N, 4.7796°E, *Rubus* sp., 4 females; 89.1-1, 89.1-2, 89.2 & 89.3 + 3 females from SFEL, Lore Vervaet; 26/6/2021, Brin-sur-Seille, 48.779761°N, 6.354036°E, *Rubus* sp., 2 females, “Darwin 3” & “Darwin 4”, Jonas Merckx; **The Netherlands**: 30/8/2021, Mook, 51.762295°N, 5.911216°E, *Vitis vinifera*, 3 females and 4 females from rearing, 172.1, Felix Wäckers; **Spain**: 2019, Monforte del Sid, *Vitis vinifera*, 3 females and 3 females from rearing, Felix Wäckers; **Italy**: 27/07/2022, Moneglia, 44°14'28.8"N, 9°29'12.3"E, *Vitis vinifera*, 5 females from rearing, 173.1, Dominiek Vangansbeke; **Morocco**: 2019, centre Agadir, feral tomato, 1 female, measured and 3 females from rearing, 171.1, Felix Wäckers; **South Africa**: 13 females from *Citrus* sp., Pobane farm, Nkwaleni Valley, Kwazulu/Natal, (28°46'S, 31°28'E), 30 January 2003, P. R. Stephen; 11 females from *Citrus sinensis* (Star Ruby), Kromhout Farm near Kakamas, Northern

Cape Province (28°47.2'S, 20°39.2'E), 13 May 2003, T. G. Grout; 13 females and one nymph from *Citrus limon* (Eureka), Zwartbosberg near Kakamas, Northern Cape Province (28°45.2'S, 20°42.3'E), 14 May 2003, T. G. Grout; 13 females from *Citrus sinensis* (Delta Valencias), Rooiduin near Kakamas, Northern Cape Province (28°42.1'S, 20°28'E), 14 May 2003, T. G. Grout; **Türkiye**: 1 female, 14/6/2016, Persimmon, Fatsa, Kılıçlı, Ordu Province, 40°59'20.39"N, 37°33'7.45"E, R. Akyazi. 1 female 22/6/2016, Persimmon, Ünye, Kadılar, Ordu Province, 41°01'56.97"N, 37°21'20.72"E, R. Akyazi. **USA CA first collection**: 1 male, 20/8/2019, orange, Lindcove, 36°21'28"N, 119°3'49"W, Y. Ouyang; 1 female, 21/8/2019, orange, Exeter, 36°17'23.40"N, 119°8'20.40"W, Y. Ouyang; 1 female, 14/8/2019, orange, Exeter, 36°17'23.40"N, 119°8'20.40"W, Y. Ouyang; 2 females, 1 male, 23/9/2020, lemon, Orosi, 36°32'19.79"N, 119°17'12"W, Y. Ouyang; 3 males, 2 females, 3 nymphs, 30/9/2020, lemon, Fresno, 36.37904°N, 119.73978°W, Y. Ouyang; ; 1 female, 21/10/2020, lemon, Orosi, 36°32'19.79"N, 119°17'12"W, Y. Ouyang; 1 male, 18/11/2020, lemon, Orosi, 36°32'19.79"N, 119°17'12"W, Y. Ouyang; **USA CA second collection**: 31/8/2021, Dinuba Fresno, 36°35'00"N, 119°20'11"W, Tangerine, Y. Ouyang, 1 female of mass rearing, 5 females of each of different SFEL, all on different slides; 15/9/2021, Fresno, 36.37904N, 119.73978W, Pomelo, Y. Ouyang, 2 females of mass rearing, 1 female of SFEL, each on different slide; 24/10/2021, Fresno, 36.60244°N, 119.50995°W, Mandarin, Y. Ouyang, 4 females of mass rearing on 1 slide; 24/10/2021, Fresno, 36.60244°N, 119.50995°W, Lemon, Y. Ouyang, 3 females of mass rearing on 1 slide, 1 female of SFEL on other slide; 15/9/2021, Fresno, 36.87904°N, 119.50995°W, Pomelo, Y. Ouyang, 7 females of mass rearing on one slide.

In this study, we found *P. ubiquitous* in California on a wide range of Citrus species (Orange, Tangerine, Lemon, Pomelo, Mandarin). In Europe, we found it on *Rubus* sp., *Rubus idaeus*, *Brugmansia* sp., *Physalis* sp., tomato, sweet pepper, cucumber, vine (*Vitis vinifera*), and fig (*Ficus carica*).

Pronematus brasiliensis n. sp.: **Brazil. First loan of material** of MZQL, ESALQ, USP, Piracicaba, Brazil used for the description: 08/05/1993, São Raimundo Nonato, PI, "Campo 12 Mata posto", I.A. Almeida, 2 female (holotype and paratype), MZQL 4476 C=2946; 08/05/1993, paratypes: São Raimundo Nonato, PI, "Campo 12 *Ipomoea* flor roxa", I.A. Almeida, 1 female, MZQL 4475 C=2945; 27/09//1991, Presidente Prudente, Instituto Biológico, *Citrus* sp., D. Botelho, 1 female, MZQL 4481 C=2951; 12/12/1996, Lavras, MG, tomato, 8 females (7 measured) and 1 protonymph, MZQL 4472 C=2942; 12/12/2001, Cananea, SP, 24°57'32"S, 47°54'34"W, dicotyledon, LVF Silva, 1 female; MZQL 11213 C=6185; 14/2/2001, Cananea, SP, 24°53'54"S, 47°50'14"W, *Tetracera oblongata*, LVF Silva, 1 male & 1 deutonymph; MZQL 11212 C=6184. **Brazil. Second loan of material** received 10/12/2023 from MZQL, ESALQ, USP, Piracicaba, Brazil, not used in the morphological study and description, all without accession numbers: 16/01/2003, Raf De Vis, 14 slides with 1 female each from the study of De Vis *et al.* (2006a); 30/08/2002 and 05/03/2003, 2 slides with respectively 1 tritonymph and one male of the study of De Vis *et al.* (2006b), ex. *Hevea brasiliensis* plantation at ESALQ (22.703168°S, 47.636764°W); 5/2/2003, Raf De Vis, 1 slides with 5 females and one tritonymph ex tomato, at 22.708408°S, 47.628912°W, ESALQ; and 18/12/2002, Raf De Vis, 3 slides each with 1 female, ex. *Passiflora edulis*, 22.715553°S, 47.641844°W, Piracicaba.

Pronematus brasiliensis n. sp. was found on wild plants such as *Ipomoea*, the climbing plant *Tetracera oblongata* or other unidentified plants, but also on crops like citrus, tomato, *Hevea brasiliensis* and *Passiflora edulis*.

Pronematus rykei: 1 female, paratype AcY65/32 at ARC-PHP (Agricultural Research Council – Plant Health Protection, Pretoria, South Africa) collected on 7/03/1964 from *Gossypium* sp. Machava, Mozambique, M.C. Rodrigues.

Pronematus karrooi: Potchefstroom, South Africa, January 1959, *Acacia karroo*, holotype (accession nr: AcY 65/28).

Pronematus tenuisetosus: holotype at ARC-PHP, Pretoria, South Africa.

Morphological redescription of *Pronematus ubiquitous***Family Iolinidae Pritchard, 1956****Subfamily Pronematinae André 1978****Genus *Pronematus* Canestrini, 1886**

Type species: *Pronematus bonatii* Canestrini 1886 (by monotypy). *Species inquirendae* sensu Kaźmierski, 1998.

As defined by Baker (1965); André (1980); Ahmad-Hosseini *et al.* (2017).

Remarks

Like Baker (1965), André (1980) included in his genus description the leg chaetotaxy, although with one difference: Baker mentioned 2 setae on femur II while André mentioned 3. Several authors reduced the description: Ahmad-Hosseini *et al.* (2017) omitted the chaetotaxy of the tibiae in their genus description. Tseng (1985) omitted also the leg chaetotaxy and mentioned “3 *ag* setae located anterior to the genital aperture” leaving doubt of the presence of a fourth *ag* seta lateral to the genital aperture, where Baker (1965) states “4 setae anterior to and laterad from the genitalia”. We suggest to retain the genus description of André (1980) except for the addition of the small seta homologous to *s* between the prorals on tarsus I, resulting in 9 setae on tarsus I (see below).

***Pronematus ubiquitous* (McGregor)**

Tydeus ubiquitous McGregor 1932: 62.

Pronematus ubiquitous (McGregor), Thor (1933): 46; Baker (1946): 255; McGregor (1956): 14; Baker (1965): 115; Meyer & Rodrigues (1966): 14; Baker (1968): 1093; Salviejo (1969): 272; Castagnoli (1984); Ueckermann & Grout (2007): 2371; Sadeghi *et al.* (2012): 110; Darbemamieh *et al.* (2020): 294; Darbemamieh *et al.* (2021): 593; Akyazi *et al.* (2022): 14. *Pronematus pruni* Meyer & Ryke, 1959: 414 sensu Meyer and Rodrigues (1966) & Kaźmierski, (1998).

Description

FEMALE (Figures 1-3). Measurements as in Table 2.

Dorsum — Prodorsum procurved, with 4 pairs of dorsal setae (*la*, *bo*, *ro*, *ex*). Dorsal face of opisthosoma with 9 pairs of setae (*c1*, *c2*, *d1*, *e1*, *f1*, *f2*, *h1*, *h2*, *ps1*). Dorsum of body completely striated. Striae longitudinally on prodorsum and between setae (*c1*) and (*d1*), whereas transversely between setae *e1* and posterior to them and longitudinal between *ps1*. Eyes absent. All dorsal setae, including bothridial setae, slender and finely serrated, relatively short, *c1* and *d1* shorter than distances between consecutive setae. Lyrifissures *ia* situated half distance between setae *c1* and *d1* and slightly laterally to imaginary line *c1-d1*. Lyrifissures *im* anterior to and closely associated with setae *e1*. Lyrifissures *ip* situated approximately half distance between *e1* and *f2*, slightly laterally to imaginary line *e1-f2*. Lyrifissures *ih* latero-ventrally associated with *h2* (Figure 1 & 2).

Venter — Striae longitudinal between metasternal setae. All ventral setae smooth and clearly shorter than dorsal setae, except for setae on the coxae which are serrate and longer. Setae *pt*, *mta* and *mtβ* almost aligned longitudinally (*mta* slightly laterally to imaginary line *pt-mtβ*). Four pairs of aggenital setae. One pair of acetabula. Setae *ps3* anterolateral of anal opening with small, striated spindle-shaped excrescence in between, not visible in specimen used for drawing but visible in other USA specimens. Epimeral formula: (3-1-4-2) (Figure 1 & 2).

Gnathosoma — Visible from above. Palptarsus eupathidium ($p\zeta$) straight and slightly forked distally. Cheliceral stylets slightly longer than palptarsus. Palp chaetotaxy (5+ ω -1-2). Subcapitulum with two subcapitular setae, *sc1-2* and two adoral setae (*ad1-2*). The adoral setae

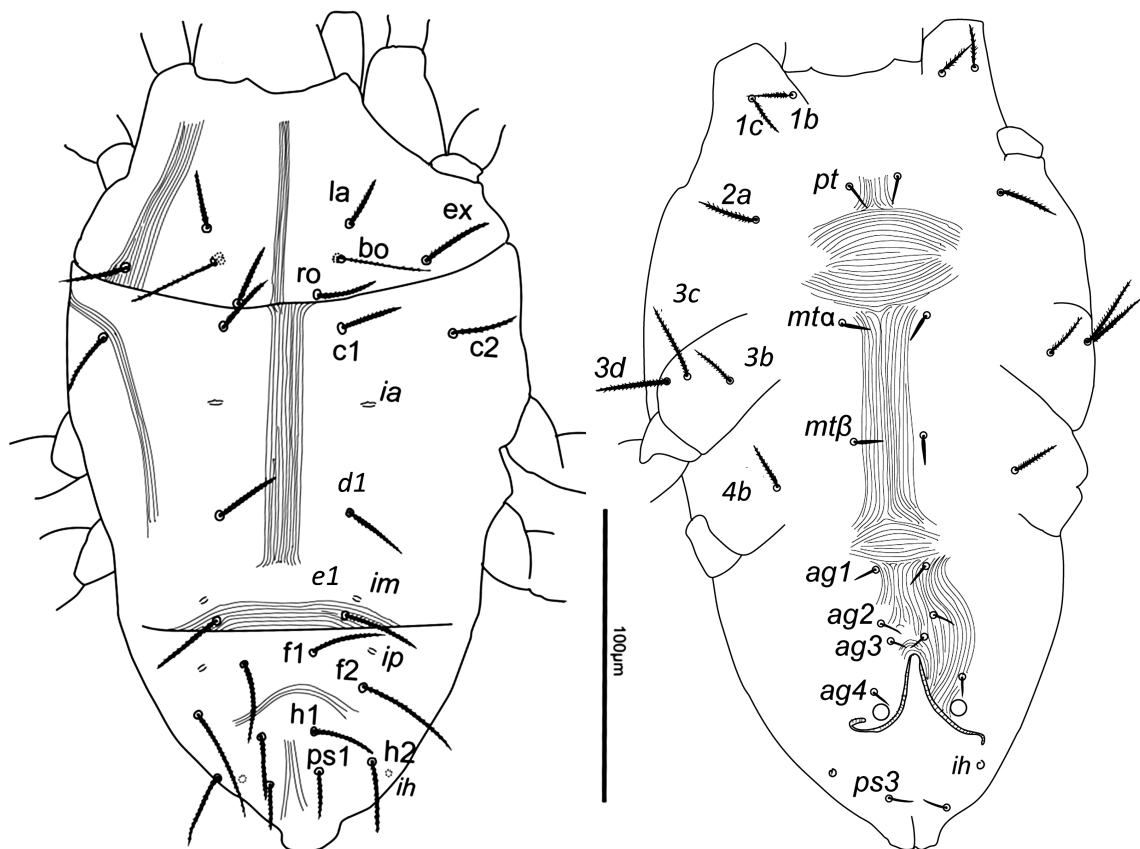


Figure 1 *Pronematus ubiquitous*, California (USA). Female dorsum (left) and venter (right).

very difficult to distinguish under phase contrast because of interference with other structures (Figure 2 & 3).

Legs — Chaetotaxy: I (9+ ω -3+k+ ϕ -3-3-0), II (6+ ω -2-3-3-0), III (5-2-2-2-1), IV (5-2-1-2-0). Femur IV not divided. Tarsus I without apotele, with 2 pairs of long serrate distal tectals and prorals ($tc'\zeta$, $tc''\zeta$, $p'\zeta$, $p''\zeta$), 1 pair of fastigials (ft' , $ft''\zeta$). Tectals and prorals plumose for all their length except for the smooth tip in most but not all specimens (smooth part up to 5 μ m). Seta $p''\zeta$ shortest. Tectals and prorals slightly sigmoid. Seta $ft''\zeta$ stout, plumose and slightly sigmoid and ft' shorter, slender, and slightly serrate. One pair of minute forked unguinals (u' , u'') and one unpaired minute seta based ventrally and between prorals, homologous to s according to Grandjean as in Evans (1992). Tarsi II–IV each with apotele, 2 claws and ciliated empodium, but without empodial claws (om) (Figures 2 & 3).

MALE (Figures 3 & 4). Measurements as in Table 3.

Idiosoma — Most characters same as in female with exception of following: dorsal setae shorter, serrate; ventral face of opisthosoma with 3 pairs of aggenital setae and $ps3$; solenidion ωI extremely long, stout (about 2 μ m at base), bent, passing anterior margin of tarsus; femora IV with a distal, abaxial, small and pointed spur (Figure 3).

TRITONYMPH Measurements as in Table 3.

Idiosomal and leg chaetotaxy like adults. Female tritonymph with 4 pairs of aggenital setae, genital pore situated medially and posterior to ($ag2$) and anterior to ($ag3$). Male tritonymph with 3 pairs of aggenital setae. Solenidion ωI of male tritonymph stouter and longer 6 than that of female tritonymph 3 (Figure 5).

DEUTONYMPH Measurements as in Table 3.

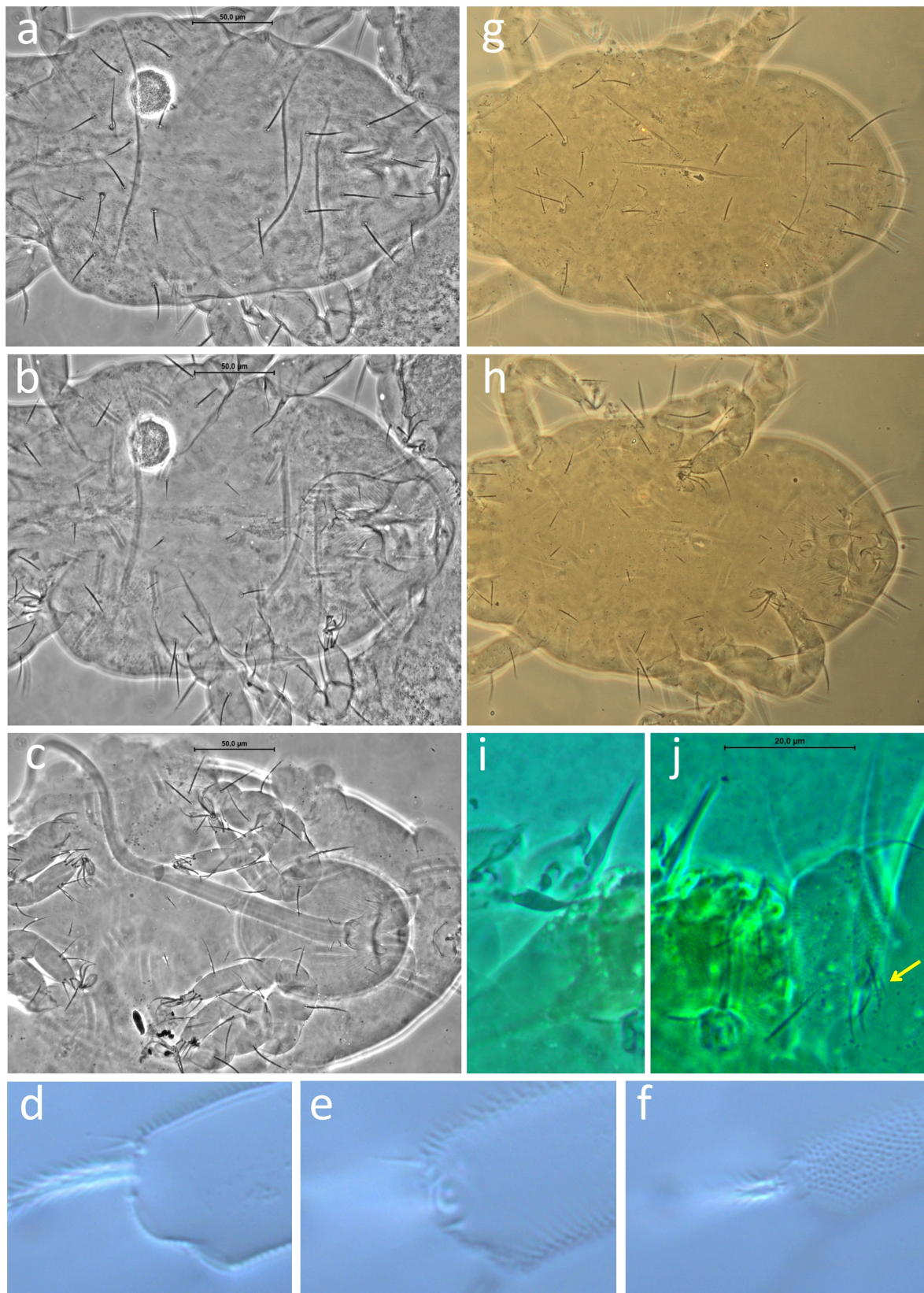


Figure 2 *Pronematus ubiquitous* California (USA) female. a – Female dorsum; b – Venter; c – Venter of female with egg stalk coming out of the genital opening; d-f – Ventral views of tip of tarsus I with different focus of exactly the same position: d – Eupathidia $p''\zeta$ with bifurcated and completely visible u'' , laciniae larger than common base, e – Seta homologous to s between bases of proral eupathidia ($p'\zeta$), f – Base of $p'\zeta$ and bifurcated u' partially visible. *Pronematus brasiliensis* n. sp.; g – Female dorsum; h – Female venter; i – Lateroventral view of chelicera with fixed and movable digit (stylet); and j – ventral view of gnathosoma palp with split dorsal seta d .

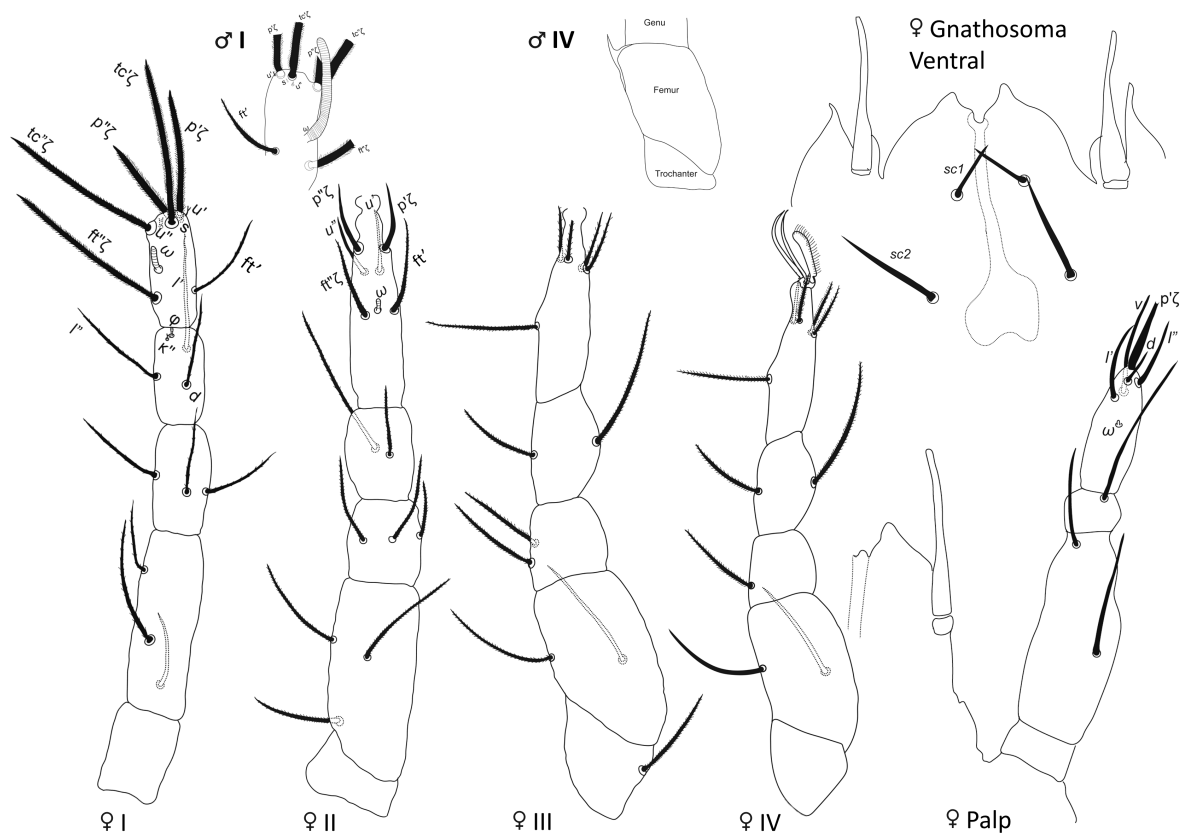


Figure 3 *Pronematus ubiquitous*, California (USA). Legs and gnathosoma with ventral and dorsal view with chelicerae, stylet, and palp.

Idiosomal chaetotaxy like female but with 2 pairs of aggenital setae, anterior to genital pore. Leg chaetotaxy like female (Figure 5). Seta *fiζ'''* on tarsus I eupathidial.

PROTONYMPH Measurements as in Table 3

Chaetotaxy of opisthosoma like female, but aggenital setae missing. Only two setae on coxae III (*3d* missing) and *mtβ* and *4b* missing. Epimeral formula 3-1-3-0 (Figure 6). Leg chaetotaxy of leg I and II like female, leg III trochanter without setae except on tarsus which is equal to female: I (9+ω-3+k+φ-3-3-0), II (6+ω-2-3-3-0), III (5-2-2-2-0), IV (5-0-0-0-0). Setae (*pζ*) and (*tcζ*) on tarsus I eupathidial, setae (*fi*) not eupathidial.

LARVA Measurements as in Table 3 (Belgian specimen).

Like protonymph except for tarsus I with small pad like empodium, without claws (*om*), setae (*p*) and (*fi*) not eupathidial, setae (*tcζ*) eupathidial, unguinal setae (*u*) and seta homologous to *s* missing. Coxae III with 1 seta (*3c* and *3d* missing), epimeral formula 3-1-2-0 (Figure 6).

Remarks

André (1981, p. 170) stated that the dorsal eupathidial setae on tarsus I of the larvae would be the prorals and the ventral setae the unguinals. But he also stated that eupathidial setae appear first as normal setae. In the larva only 2 eupathidial dorsal setae are present. The ventral setae are normal setae and larger (7,6 – 9) than the length of the unguinal setae in the adult. The fastigial setae are normal. In the protonymph, 4 eupathidial setae ((*tcζ*), (*pζ*)) are present with (*fi*) normal and in the deutonymph, 5 eupathidial setae ((*tcζ*), (*pζ*) and *fi'''ζ*) are present with *fi'* normal, as in adult. We believe therefore that the ventral setae in the larva are not the unguinal

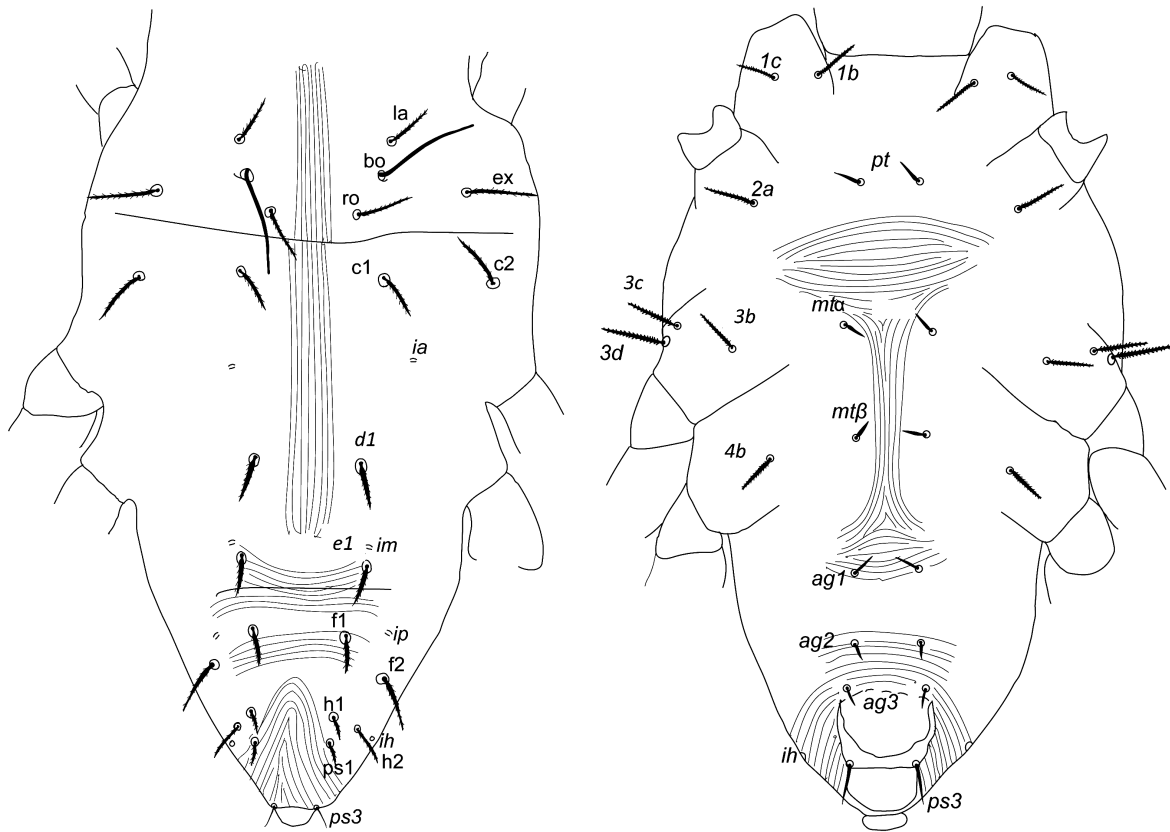


Figure 4 *Pronematus ubiquitous*, California (USA). Male dorsum (left) and venter (right).

Morocco, a lab colony (171) was set up by Biobest NV, 3 SFEL (171.1-3) could be established, and 1 DNA extraction (171.1) was successful from mites preserved in 90% alcohol. The names or numbers refer to the sample reference (see above, in material studied). A total of 25 COI sequences were obtained.

After cleaning, we used a 657 bp fragment of the COI for alignment. No insertions or deletions were found among the compared sequences and we found 6 distinct sequences among the *P. ubiquitous* sequences. The translation of the mtDNA COI nucleotide sequences resulted in a polypeptide of 219 amino acids in length. Alignment of these amino acid sequences revealed that the *P. ubiquitous* sequences were completely identical. As is generally the case in insects and mites, base pair frequencies showed that the region was A+T-rich. The G+C and A+T composition of the entire data set ranged from 20.70 to 36.07% and 63.93 to 79.30%, respectively. For *P. ubiquitous* this was 30.56 to 31.66% and 68.34 to 69.41%, respectively. A Blast search of the Genbank database showed that the sequences blasted with other mite COI sequences of, for example, *T. urticae*. There are no *P. ubiquitous* sequences available in the GenBank yet. See Figure 7 for the Maximum likelihood consensus tree.

Comparison of different origins and principal component analysis (PCA)

In a first run of the PCA, all 42 parameters were used but we could not find a good separation between the different origins. Leg length, body length and body width came out as principal components (figure not shown). These parameters are susceptible to measurement errors as the legs or the body are sometimes bent, they can be stretched or pushed up thus influencing the measurement. In a second run, excluding the previous mentioned parameters, we concluded the same for distances between setae. As *Pronematus* lacks shields or sclerotised structures,

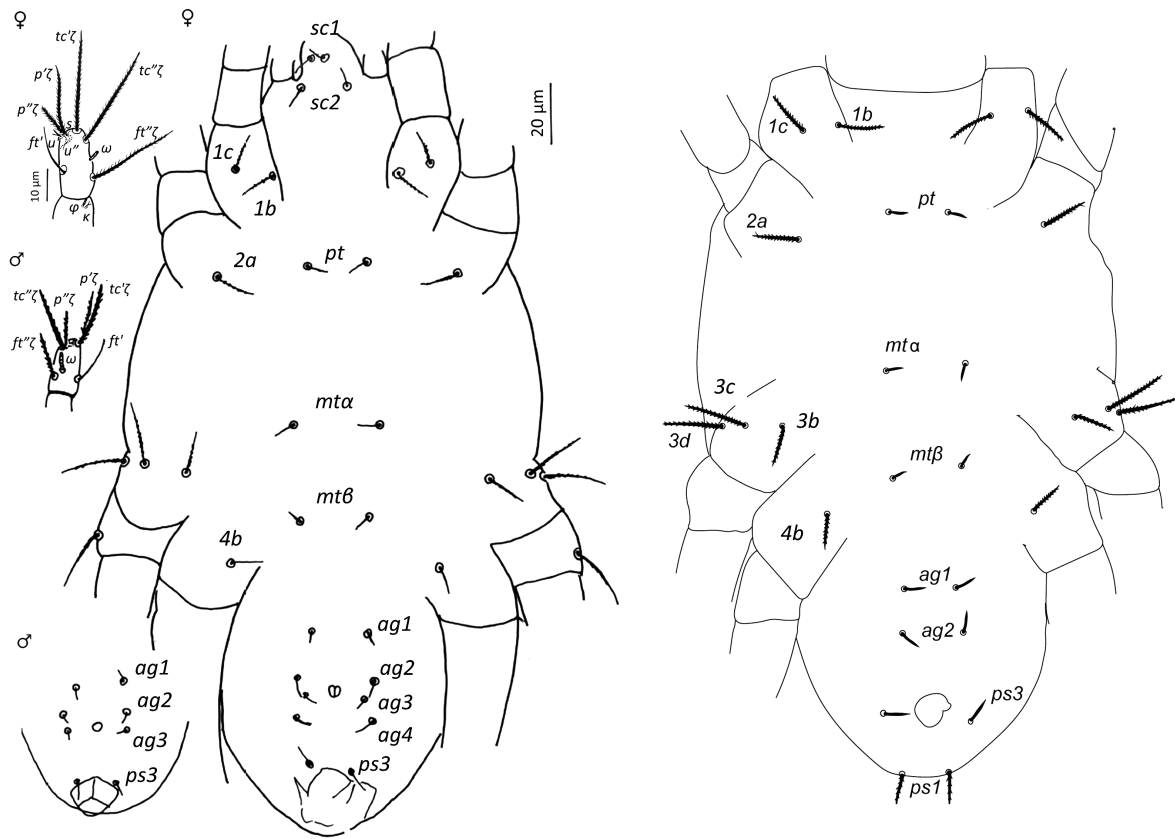


Figure 5 *Pronematus ubiquitous* California (USA), venter and tarsus I of female and male tritonymph (left) and venter of deutonymph (right). Drawings of male tritonymph of a Belgian specimen.

setal distances are also susceptible to the effect of mounting conditions, the size or feeding conditions of the mite and/or the presence of an egg in the body of the female. In the third run, also excluding distances, we observed separation of certain origins. The loading of axis 1 of the dorsal setae was highest with *ro*, *c1*, *c2*, *d1*, *e1*, *f1*, *f2* and *3d* having a loading higher than 0.2, followed by the eupathidia on tarsus I and ventral setae *2a*, *3b*, *3c*, and *3d*. Still, certain parameters were characterized by a low loading such as the other ventral setae (Figure 8A).

The Californian (USA) and African specimens clustered apart from the European specimens, albeit with some overlap. All European specimens clustered together with considerable overlap of the specimens originating from the different European countries. The South African specimens overlapped with the specimens from California (USA), but the Moroccan specimens separated from these specimens, again with some overlap. We therefore consider the specimens of all these countries as *P. ubiquitous*. The mite specimens originating from Brazil separate without overlap from those from all other geographical regions (Figure 8B). We thus consider them as a distinct species, described below.

Our study showed a clear relation of the length of *e1* with latitude where the specimens were collected (Figure 9). Longer *e1* lengths were found in specimens that were collected at higher latitudes.

***Pronematus brasiliensis* De Vis & Ueckermann n. sp.**

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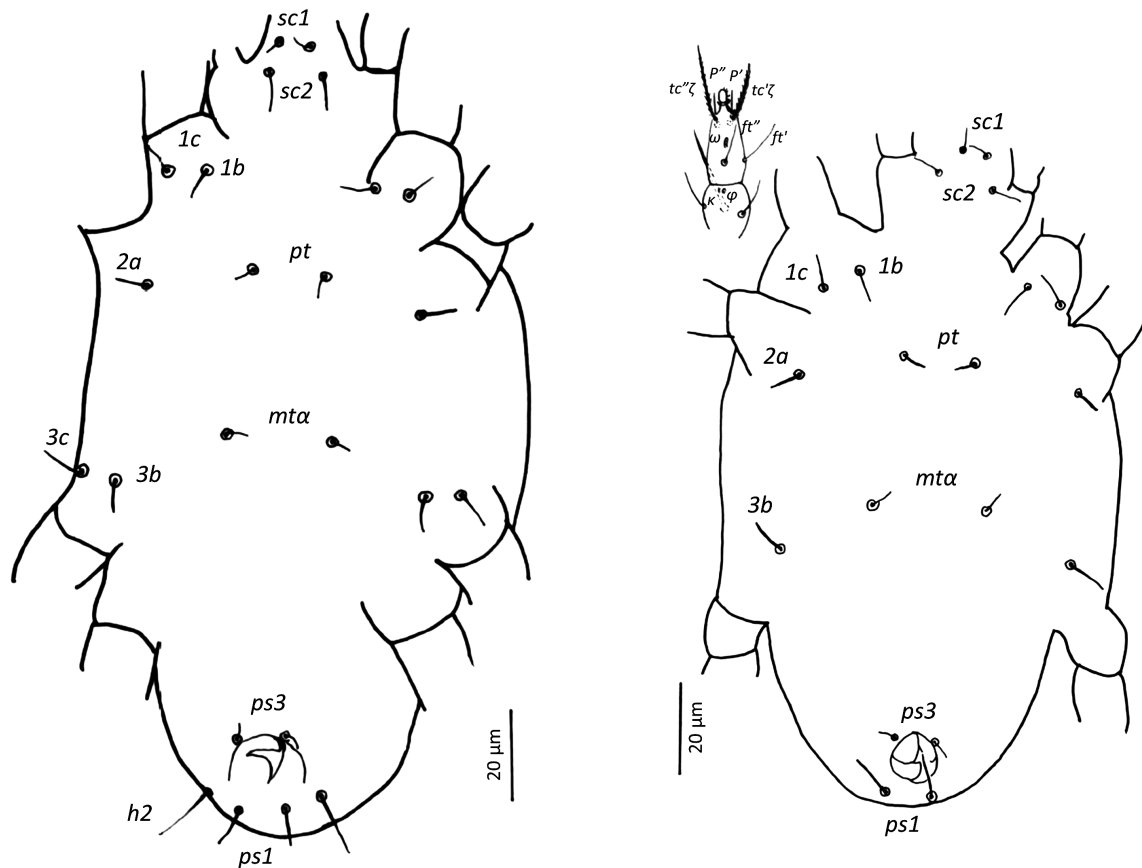


Figure 6 *Pronematus ubiquitous*, venter of protonymph, California (USA) (left) and venter and tarsus I of larva, Belgium (right).

Description

FEMALE Measurements as in Table 3.

Dorsum — Prodorsum procurved, with 4 pairs of dorsal setae (*la, bo, ro, ex*). Dorsal face of opisthosoma with 9 pairs of setae (*c1, c2, d1, e1, fl, f2, h1, h2, ps1*). Dorsum of body completely striated. Striae longitudinally on prodorsum, but circular around prodorsal setae; forming V to U on central part of dorsal face of the opisthosoma, making turn between or around setae (*e1*) and (*fl*); transversely between setae *e1* and posterior to it and making inverted V between *ps1*. Eyes absent. All dorsal setae, including bothridial setae, slender and finely serrated, relatively short, *c1* and *d1* shorter than distances between consecutive setae. Lyrifissures *ia* situated half distance between setae *c2* and *d1* and slightly laterally to imaginary line *c2-d1*. Lyrifissures *im* anterior to and closely associated with setae *e1*. Lyrifissures *ip* situated approximately half distance between *e1* and *f2*, slightly laterally to imaginary line *e1-f2*. Lyrifissures *ih* latero-ventrally associated with *h2* (Figure 10).

Venter — Completely striated. Striae longitudinal between metasternal setae, between *pt* and *mta* forming spindle broader than distance between (*mta*) but smaller than distance between setae *2a*, forming second spindle distally anterior to setae *ag1*, about double as broad as distance between setae *ag1*. All ventral setae smooth and clearly shorter than dorsal setae, except for setae on coxae which are serrate and longer. Setae *pt, mta* and *mtβ* almost aligned longitudinally (*mta* slightly laterally to imaginary line *pt- mtβ*). Four pairs of aggenital setae. One pair of acetabula, with smaller and circular unstriated zone anterior to them (function unknown). Setae *ps3* anterolateral of anal opening with small, spindle-shaped and striated

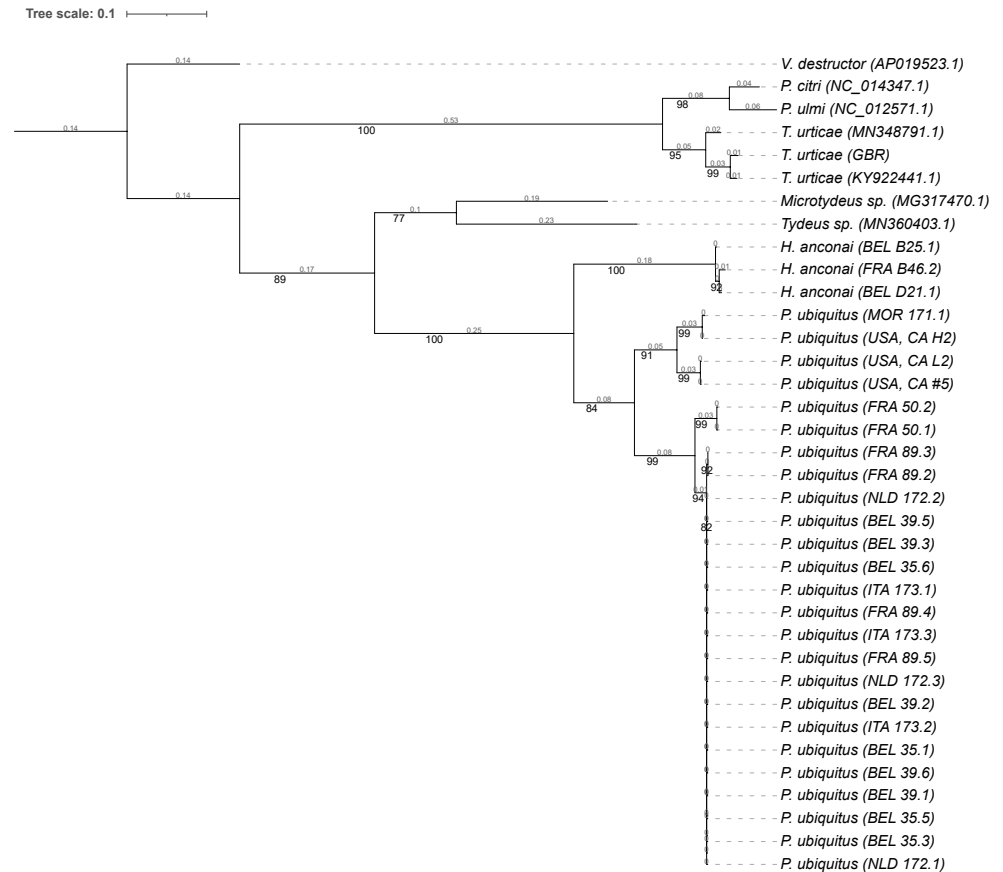


Figure 7 Maximum likelihood consensus phylogenetic tree of *Pronematus ubiquitous* and gradually less related species. Bootstrap values and branch lengths are shown respectively below and above the branches.

excrescence in between. Epimeral formula: (3-1-4-2) (Figure 10).

Gnathosoma — Visible from above. Palptarsus eupathidium $p\zeta$ straight and slightly forked distally. Fixed cheliceral digit pointed, triangular spine; cheliceral stylet slightly longer than palptarsus. Palp chaetotaxy (5+ ω -1-2). Dorsal seta *d* of palptarsus forked. Subcapitulum with two subcapitular setae, *scl*-2 and two adorals (*adl*-2) (Figure 11).

Legs — Chaetotaxy: I (9+ ω -3+*k*+ φ -3-3-0), II (6+ ω -2-3-3-0), III (5-2-2-2-1), IV (5-2-1-2-0). Femur IV not divided. Tarsus I without apotele, with 2 pairs of long serrate distal dorsal tectals and ventral prorals ($tc'\zeta$, $tc''\zeta$, $p'\zeta$, $p''\zeta$), 1 pair of fastigials (ft' , $ft''\zeta$), 1 pair of minute, bifurcate unguinals (u' , u'') and minute seta, homologous to *s* between prorals. All tectals and prorals plumose for all their length except for smooth tip. Seta $p''\zeta$ shortest. Tectals and prorals slightly sigmoid. Seta $ft''\zeta$ stout and plumose and ft' shorter, slender, and slightly serrate. Tarsi II–IV each with apotele, 2 claws and ciliated empodium, but without empodial claws (*om*) (Figure 11).

MALE Measurements as in Table 3.

Most of characters same as in female with exception of following: smaller than female, dorsal setae shorter, clearly serrate; 3 *ag* setae; adaxial spine on genu IV; ωl 16.5 very stout, tapering from basis to tip, 2.5 broad at basis, 1.8 middle and 0.9 at tip. Setae (*u*) forked, 2.2-2.9, seta homologous to *s* 1.6-2.5, palptarsal seta *d* forked (Figure 11 & 12).

TRITONYMPH (male)

Idiosomal and leg chaetotaxy like adults. Legs: I (9+ ω -3+*k*+ φ -3-3-0), II (6+ ω -2-3-3-0), III (5-2-2-2-1), IV (5-2-1-2-0). Solenidion ωl 2.8. Femur IV not divided. with 3 pairs of aggenital

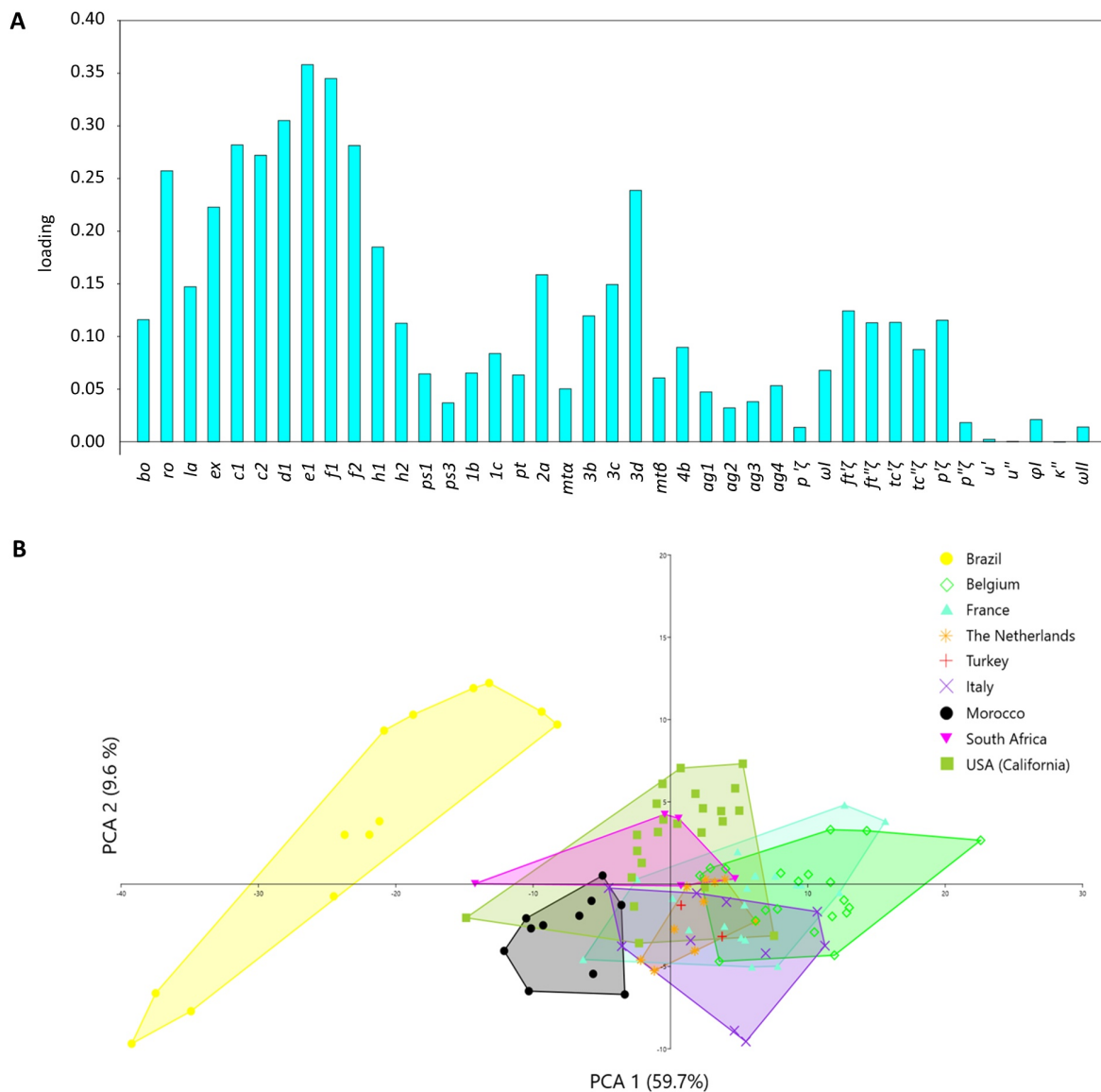


Figure 8 Principal component analysis (PCA) of morphometric characters measured on females of *Pronematus ubiquitus* populations from distinct regions. A – loading of axis 1 for different characters B – Polygon graph. Each polygon refers to a different origin.

setae, genital pore situated medially and posterior to (*ag2*) and anterior to (*ag3*). Bifurcation of palpdorsal seta *d* not distinguishable. Eupathidia on tarsus I like in female.

DEUTONYMPH

Idiosomal and leg chaetotaxy like adults. Leg chaetotaxy: I (9+ω-3+k+φ-3-3-0), II (6+ω-2-3-3-0), III (5-2-2-2-1), IV (5-2-1-2-0). Femur IV not divided. Bifurcation of palpdorsal seta *d* not distinguishable. 2 *ag* setae. Five eupathidial setae ((*tc'ζ*), (*p'ζ*) and *ft'ζ''*) are present on tarsus I with *ft'* normal; like in female.

PROTONYMPH

Leg chaetotaxy: I (9+ω-3+k+φ-3-3-0), II (6+ω-2-3-3-0), III (5-2-2-2-1), IV (5-0-0-0-0). Femur IV not divided. Bifurcation of palpdorsal seta *d* not distinguishable. No *ag* setae. Four eupathidial setae ((*tc'ζ*), (*p'ζ*)) are present on tarsus I with (*ft*) normal.

LARVA not available.

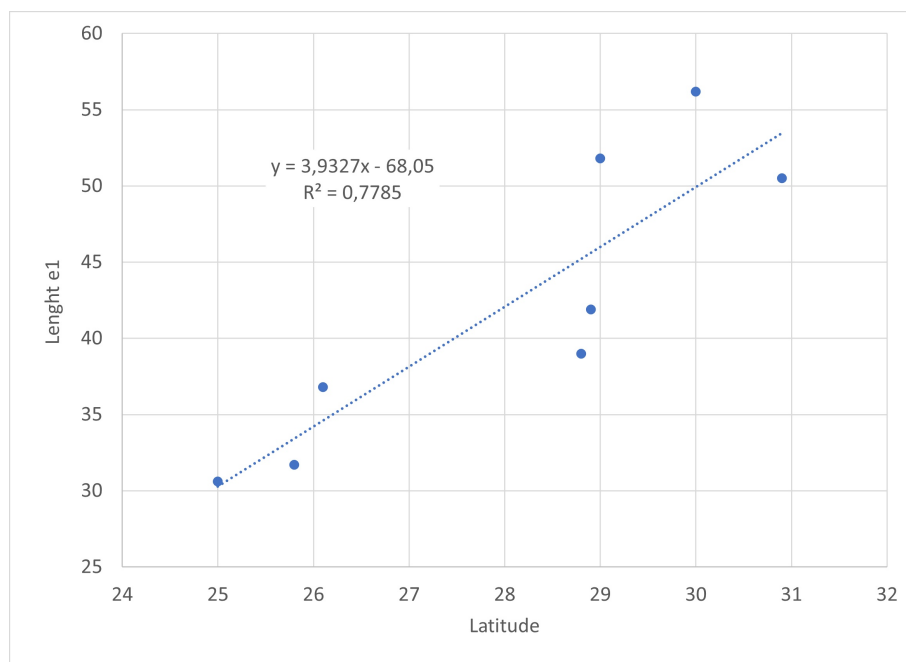


Figure 9 Length (μm) of seta e1 of *Pronematus* spp. as influenced by the latitude where they were found.

Type material

See material studied for the information on the type series.

Differential diagnosis

Compared to *P. ubiquitous* with *c1* 19-24, *c2* 19-24, *d1* 19-26, *e1* 22-28 and *fl* 23-30, *P. brasiliensis* **n. sp.** has shorter dorsal setae with *c1* 14-16, *c2* 15-18, *d1* 15-18, *e1* 15-21 and *fl* 15-24, reason why *P. ubiquitous* separates from *P. brasiliensis* **n. sp.** in the PCA analysis (Figure 8B). Additionally, it has palptarsal dorsal seta *d* forked (not always visible).

Pronematus juglandi **n. sp.** has *c1* 21-22, *c2* 21-22, *d1* 21-23, *e1* 24-26 and *fl* 25-27 longer than *P. brasiliensis* **n. sp.** and *P. rykei* has *c1* 27.1, *d1* 26.8, *e1* 27 longer than *P. brasiliensis* **n. sp.**. It resembles *P. rykei* in having palpdorsal seta *d* forked. It resembles *P. perpusillus* in having dorsal setae short but *P. perpusillus* has dorsal setae lanceolate. See also Table 4 and key below.

The male characteristics of *P. ubiquitous* and *P. brasiliensis* **n. sp.** are similar, except for $\omega 1$ which is stouter and broader at its base for *P. brasiliensis* **n. sp.**.

Pronematus rykei Meyer & Rodrigues, 1966

Pronematus rykei. Meyer and Rodrigues 1966: 20.

Pronematus rykei. Ahmad-Hosseini *et al.* 2017: 499. Misidentification

FEMALE Measurements of the paratype (accession nr AcY65/33) as in Table 5.

Redescription

Dorsum — (Figure 13). Prodorsum procurved, with 4 pairs of dorsal setae (*la*, *bo*, *ro*, *ex*). Eyes absent. Dorsal face of opisthosoma with 9 pairs of setae (*c1*, *c2*, *d1*, *e1*, *fl*, *f2*, *h1*, *h2*, *ps1*). Dorsum of body completely striated. Because of the condition of the paratype striae are mostly indistinct. Striae apparently longitudinally on prodorsum, but clearly longitudinal

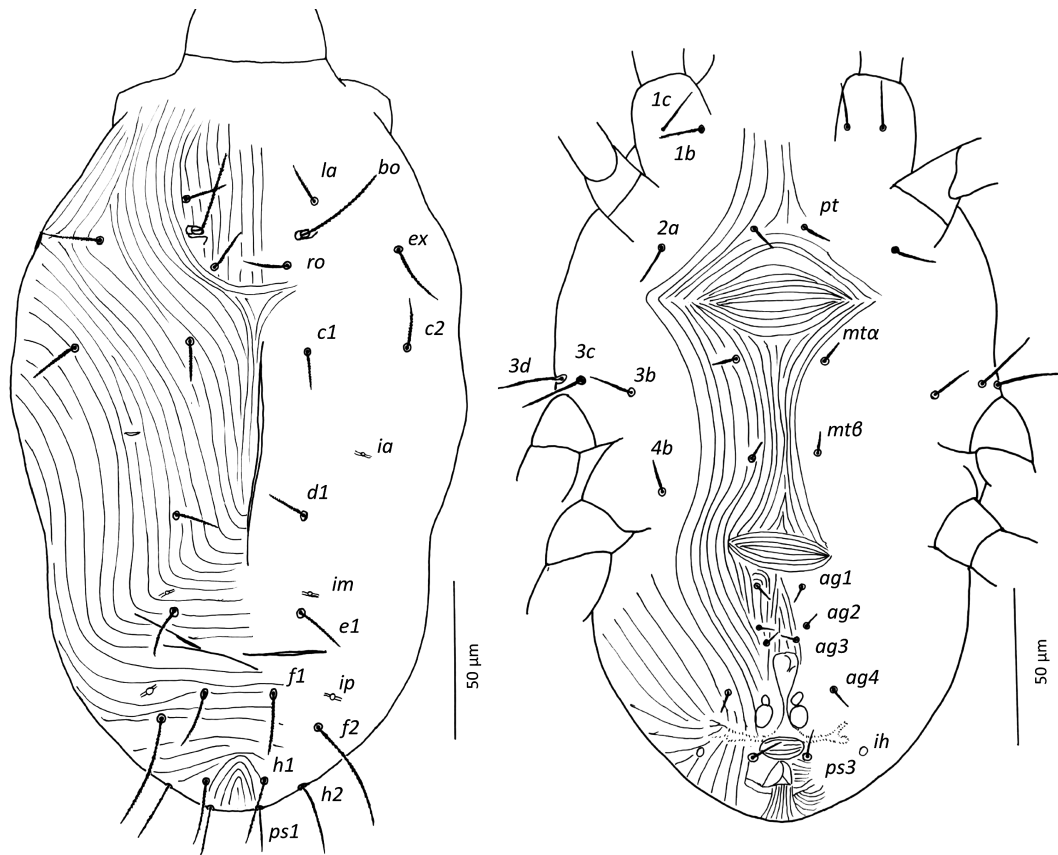


Figure 10 *Pronematus brasiliensis* n. sp.. Female (holotype) dorsum (left) and venter (right).

on central part of dorsal face of opisthosoma, between setae (*c1*) and (*d1*), and transversely between setae *e1* and posterior to it and probably longitudinal between *ps1*. All dorsal setae, including bothridial setae, slender and finely serrated, relatively short, *c1* and *d1* shorter than distances between consecutive setae. Lyrifissures not visible in paratype.

Venter — (Figure 13). Striae longitudinal between metasternal setae. Spindle formed by striae only visible between setae *pt* and *mta*. All ventral setae appeared plumose and clearly shorter than dorsal setae. Setae *pt* and *mtb* almost aligned longitudinally, but *mta* clearly further apart than *pt* and *mtb*. Four pairs of plumose aggenital setae. Acetabula not visible. Pseudanal valves with 1 pair of setae (*ps3*). Epimeral formula (3-1-4-2).

Table 4 Characterisation of the *Pronematus ubiquitus* COI-sequences as deposited in the Genbank database.

Sequence ID	Collector	Collection date (dd/mm/yyyy)	Country	Latitude, Longitude	Isolation source	Isolate	Voucher specimen source	Mounting date (dd/mm/yyyy)	Slide label *	GenBank accession number
Seq1	Yuling Ouyang	2021-08-31	USA California	Dinuba 36.583230, -119.336418	<i>Cirus x aurantium</i> Tangerine	CAL_#7	single female line # 7	2021-09-18	CBGP ACARI 2486	PP090881
Seq2	Yuling Ouyang	2021-10-24	USA California	Fresno 36.602442, -119.510995	<i>Citrus limon</i> Lemon	CAL_L2	single female line L2	2021-12-01	CBGP ACARI 2487	PP090882
Seq3	Yuling Ouyang	2021-09-15	USA California	Fresno 36.87904, -119.73978	<i>Cirus x aurantium</i> Pomelo	CAL_H2	single female line H2	2021-11-05	CBGP ACARI 2488	PP090883
Seq4	Felix Wäckers	2021-08-30	The Netherlands	51.762289, 5.911175	<i>Vitis vinifera</i>	NLD_172.1	mass rearing	2021-11-02	CBGP ACARI 2489	PP090884
Seq5	Felix Wäckers	2019-07-01	Morocco	30.424302, -9.597646	<i>Solanum lycopersicum</i> Feral tomato	MOR_171.1	mass rearing	2020-06-23	CBGP ACARI 2490	PP090885
Seq6	Dominiek Vangansbeke	2022-07-27	Italy	44.241326, 9.486696	<i>Vitis vinifera</i>	ITA_173.3	single female line	2023-06-09	CBGP ACARI 2491	PP090886
Seq7	Lore Vervaeet	2018-10-17	France	44.5120, 4.7796	<i>Rubus</i> sp.	FRA_89.2	single female line 89.2	2019-04-16	CBGP ACARI 2492	PP090887
Seq8	Dieter Foqué	2018-09-29	France	46.362778, 2.349444	<i>Capsicum annuum</i> Sweet pepper	FRA_50.1	single female line 50.1	2018-12-14	CBGP ACARI 2493	PP090888
Seq9	Raf De Vis	2018-09-27	Belgium	50.866798, 4.159654	<i>Brugmansia</i> sp.	BEL_39.1	single female line 39.1	2019-04-12	CBGP ACARI 2494	PP090889
Seq10	Eva Reybroeck	2018-09-27	Belgium	50.945049, 3.487808	<i>Cucumis sativus</i> Cucumber	BEL_35.1	single female line 35.1	2018-12-14	CBGP ACARI 2495	PP090890

*Collection Centre de Biologie pour la Gestion des Population, Univ Montpellier, CIRAD, INRAE, Institut Agro, IRD, Montpellier, France "CBGP - Continental Arthropod Collection", <https://doi.org/10.15454/D6XAKL>

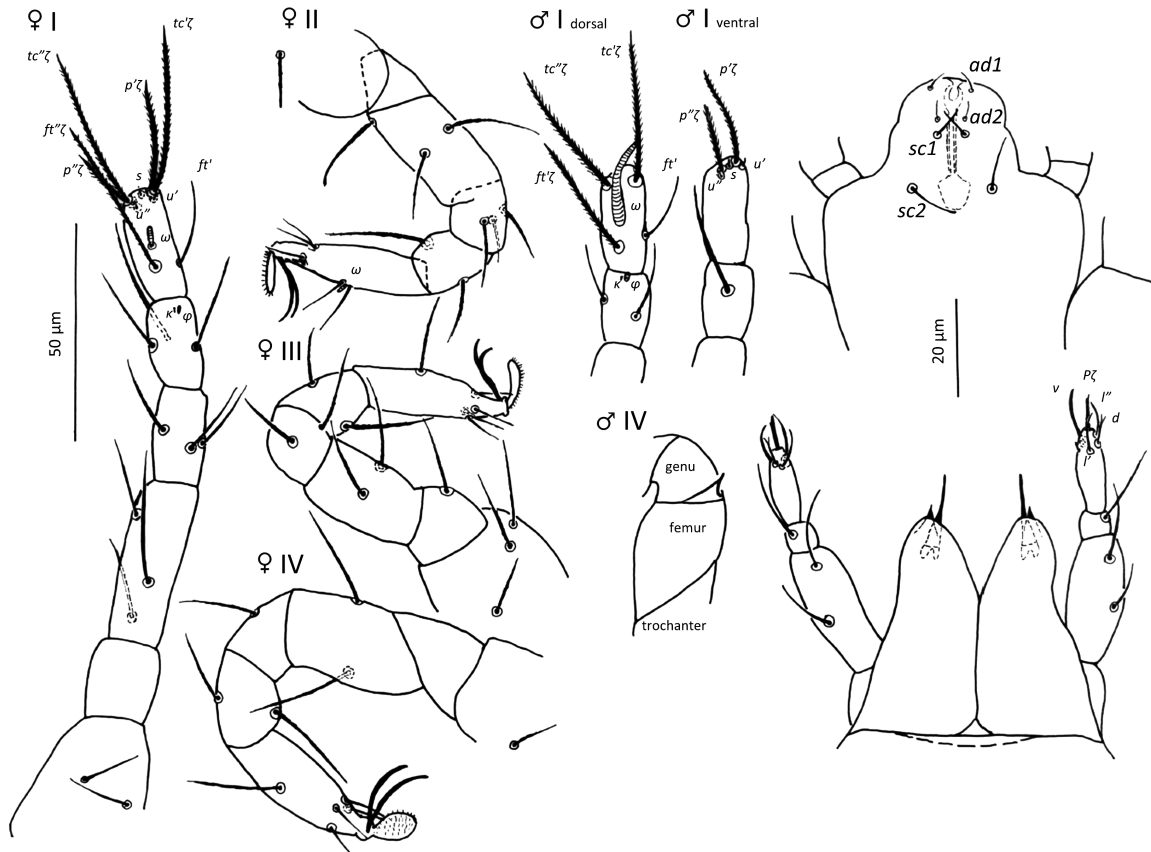


Figure 11 *Pronematus brasiliensis* n. sp.. Female (holotype): leg I-IV, gnathosoma ventral view with *sc* and *ad* setae and dorsal view with chelicerae with fixed and movable digit, and palp with forked dorsal seta *d*. Male (paratype): leg I dorsal view with large, tapering ωl and ventral view with (*u*) and *s*; leg IV dorsal view right femur with adaxial spur.

Gnathosoma — (Figure 14). Dorsal view. Palptarsus eupathidium ($p\zeta$) straight and slightly acute distally. Seta *d* forked. Cheliceral stylets slightly longer than palptarsus. Palp chaetotaxy (5+ ω -1-2). Subcapitulum with two subcapitular setae, *sc1*-2, and two adorals *ad1*-2.

Legs — (Figure 14). Chaetotaxy: Legs I, II and IV broken off in paratype but trochanteral formula (0-0-1-0). Chaetotaxy of leg III (5-2-2-2-1). According to Meyer and Rodrigues (1966), tarsus I without apotele, with 2 pairs of long serrate distal tectals and prorals ($tc'\zeta$, $tc''\zeta$, $p'\zeta$, $p''\zeta$), 1 pair of fastigials (ft' , $ft''\zeta$). One pair of minute forked unguinals (u' , u'') and unpaired seta homologous to *s* between unguinals obviously not indicated by Meyer & Rodrigues (1966). They depicted tectals and prorals as serrate. Tarsi II–IV each with apotele, 2 claws and ciliated empodium, but without empodial claws (*om*).

Type material — Mozambique: 17/04/1964, R. Quetxoio, *Gossypium* sp., one paratype (accession nr AcY65/33), ARC-PHP, Pretoria, South Africa.

Remarks

Only one paratype remains in the collection of ARC-PHP, Pretoria, South Africa and was available for study within the scope of this study. The other 5 female types (including holotype) were sent to Instituto do Algodão de Mozambique, Maputo, Mozambique, and the National Parks Board of South Africa.

Meyer and Rodrigues (1966) stated in their description that the distinguishing character of *P. rykei* when compared with *P. ubiquitous* are the longer dorsal setae. When we compare the measurements of the paratype of *P. rykei* with those of our Californian (USA) specimens of *P.*

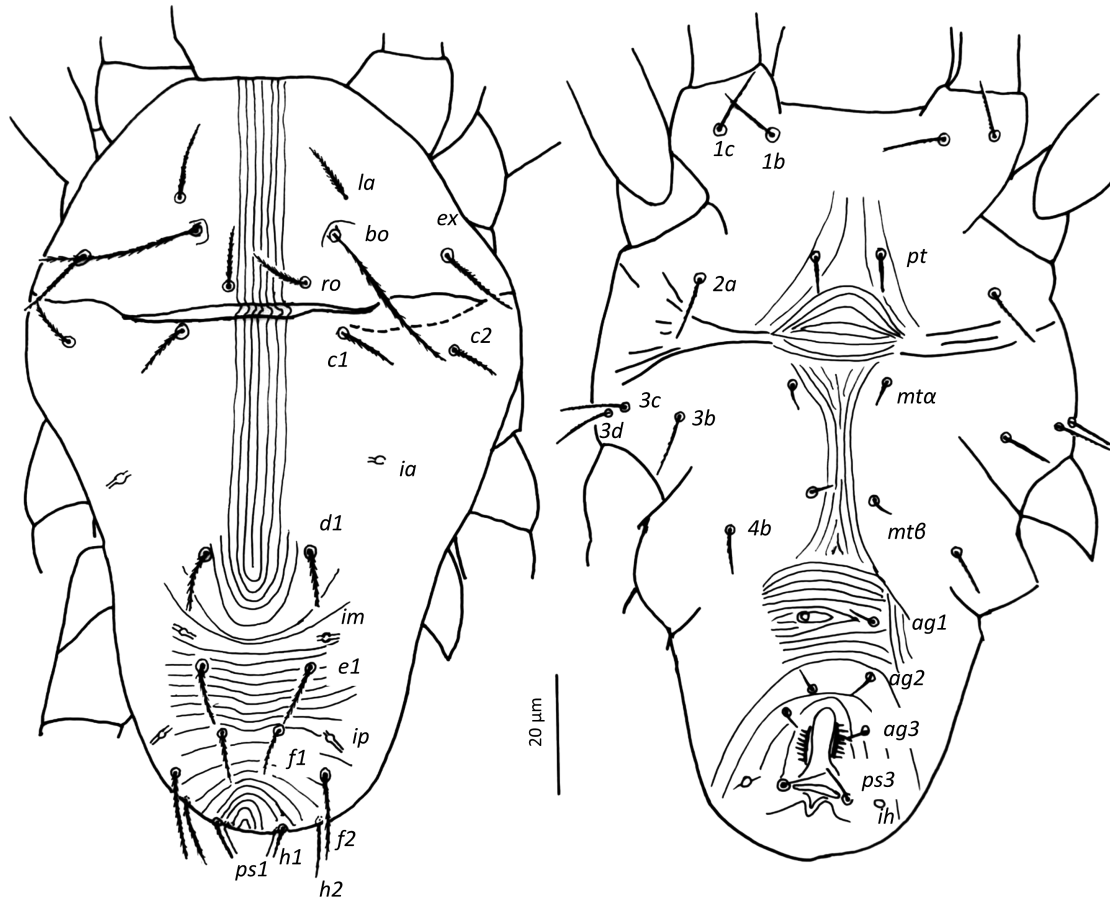


Figure 12 *Pronematus brasiliensis* n. sp.. Male (paratype) dorsum (left) and venter (right).

ubiquitus, several setae of the paratype fall within the range of *P. ubiquitus*, except for *la*, *ex*, *c1*, *d1* which are indeed longer, but *f1* 21,5, *h1* 18.4 on the other hand, are shorter (Table 5). In the same publication, they report findings of *P. ubiquitus* in several countries of southern Africa (Mozambique, South Africa, and Zambia), and they also mention the variability in setal length of *P. ubiquitus*.

Meyer and Rodrigues (1966) mention the absence of a seta on trochanter III but we confirm the presence of one seta here (Figure 14), which confirms it is a *Pronematus* species. Also, they mention 4 setae on tibia I (normally $3+\kappa''+\phi$). We suppose that κ'' was not considered, we could not confirm this because the paratype lacks legs I and II.

Ahmad-Hosseini *et al.* (2017) redescribed *P. rykei* but their measurements do not match with those of the paratype of *P. rykei* (Table 5). Additionally, as they also state, their specimens are different in not having palptarsal seta *d* forked. We therefore rename the specimens described by them as new species, described below.

***Pronematus juglandi* De Vis & Ueckermann n. sp.**

Zoobank: 16A3CA7E-D451-4813-AC75-1A3C7BBFD90E

Pronematus rykei Ahmad-Hosseini *et al.* 2017: 499.

Complete description as in Ahmad-Hosseini *et al.* (2017) with the corrections that the length of the setae $p'\zeta$ and $p''\zeta$ have to be switched as also the name of those setae on the drawing.

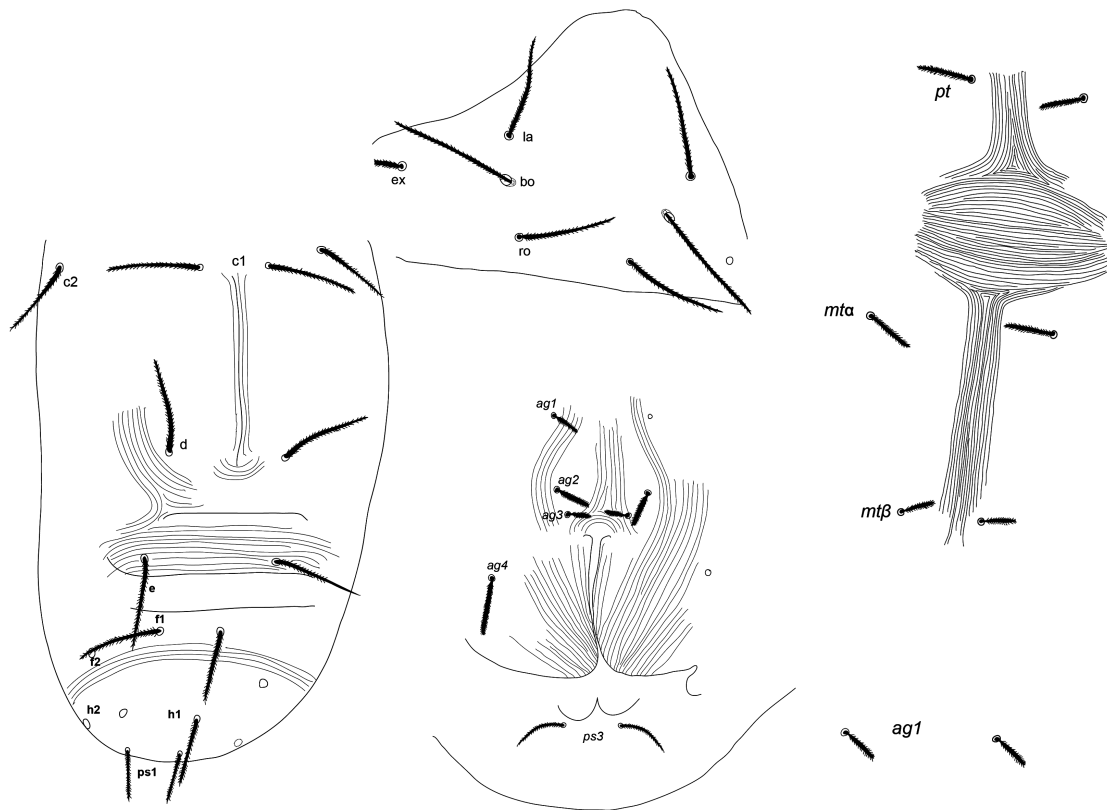


Figure 13 *Pronematus rykei*. Female prodorsum (upper side, centre), opisthosoma (left), genital area (middle) and venter (right).

Etymology

The specimens were collected by Ahmad-Hosseini *et al.* (2017) from leaves of walnut trees, *Juglans regia* L. (Juglandaceae), infested by leaf gall mite, *Aceria tristriatus* Nalepa, Eriophyidae in different regions in Iran.

Remarks

Pronematus juglandi **n. sp.** has setae *la* 15-16, *c1* 21-22 and *d1* 21-23 considerably shorter but has *f1* 25-27, *1b* 20-22, *1c* 19-20 and *2a* 21-22 considerably longer than the paratype of *P. rykei* we measured. Other deviations from the original description of *P. rykei* as described above.

Distinguishing characters

Pronematus juglandi **n. sp.** resembles *P. ubiquitous* but has dorsal seta *h1* 25-27 and ventral setae *1b* 20-22 and *1c* 19-20 longer than in the Californian (USA) *P. ubiquitous* we measured (Table 2). Additionally, we do not see a spindle of striae near the *ag1* setae on Figure 16 on p. 502 of Ahmad-Hosseini *et al.* (2017). Instead, the proximal striae make a turn near/around the *ag1* setae, and the distal striae make a turn around *ag2* and *ag3* setae.

Type material

For the moment, no types have been defined. The specimens used for the redescription are deposited in the mite collection of the Acarology Laboratory of the BuAli Sina University, Hamedan, Iran. We do not have access to those specimens and the designation of the type series can only be done by one of the authors of Ahmad-Hosseini *et al.* (2017).

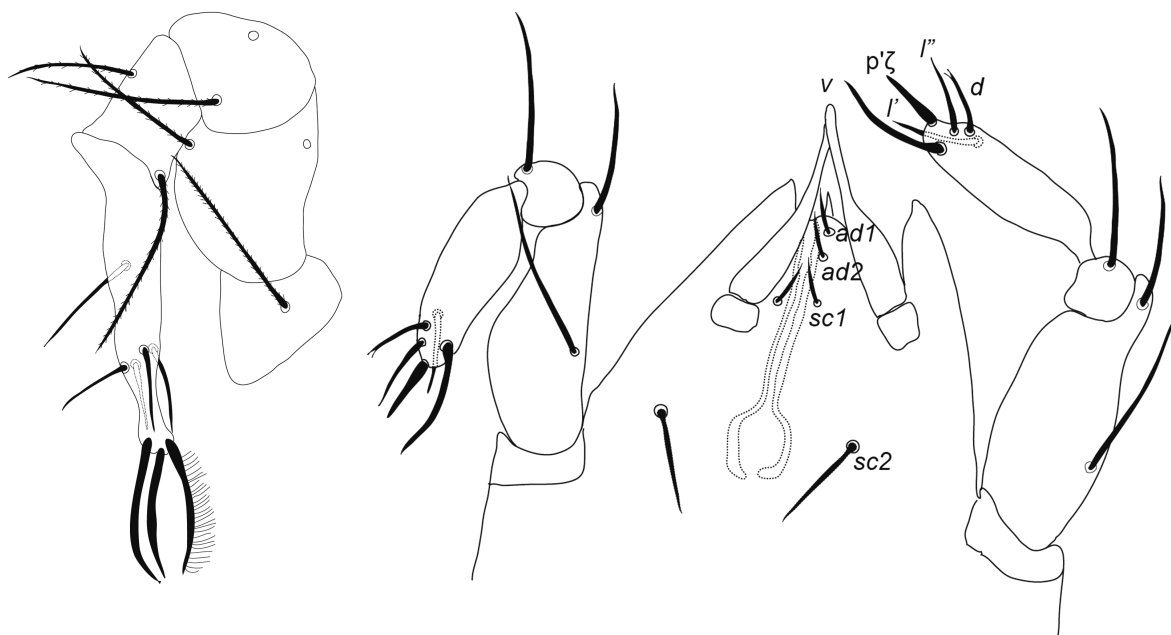


Figure 14 *Pronematus rykei*. Female leg III and gnathosoma.

***Pronematus karrooi* Ryke & Meyer, 1960**

Pronematus karrooi Ryke and Meyer 1960: 568.

Female. New measurements of the holotype as in Table 5. Ryke and Meyer (1960) described *P. karrooi* having *bo* 27, tarsus I 29 and dorsal setae 21-25 (*ωI* not mentioned). With these characters we can distinguish them from other species.

Redescription

Unfortunately, the holotype is in bad condition and only few details can be added to the original description. We recommend collecting more material at the original site and make a detailed description, as to confirm the differential characters described below.

Distinguishing characters

Our measurement of *bo* 28.4 is slightly higher than that of the original description (27). This is still shorter than the minimal length of 30.8 of the Californian (USA) and European specimens of *P. ubiquitous* (Table 2). Furthermore, *f2* 25.3 and *h1* 17 are shorter than in *P. ubiquitous*. The other setal lengths fall within the range of *P. ubiquitous*.

Type material

Potchefstroom, South Africa, January 1959, *Acacia karroo*, holotype (accession nr : AcY 65/28), ARC-PHP, Pretoria, South Africa.

***Pronematus* species review and key for the confirmed species**

Kaźmierski (1989) listed tree confirmed species. André (2021) actualized the list on the wikispecies website and counted 11 species (accessed on 08/2023). Revising the descriptions, we consider the following:

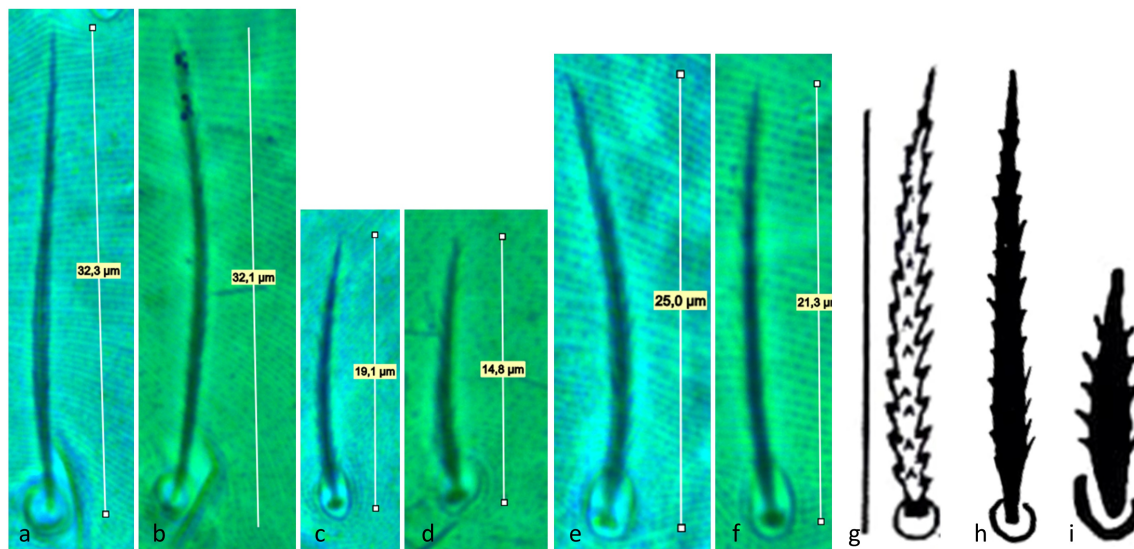


Figure 15 Dorsal setae of *Pronematus* spp. Bothridial seta bo: a – *P. ubiquitous*, b – *P. brasiliensis* n. sp.. Seta ro: c – *P. ubiquitous*, d – *P. brasiliensis* n. sp.. Seta fl: e – *P. ubiquitous*, f – *P. brasiliensis* n. sp., g – *P. debilis* (bar = 20 μm, after Tseng (1985)), h – *P. perpusillus* 17 μm (after Tseng (1985)). A dorsal seta: i – *P. oryzae* – (after Menon *et al.* (2007)).

• Tseng (1985) described *P. debilis* and *P. perpusillus* including the leg chaetotaxy. The species description states “The *debilis* differs from all known *Pronematus* by having 3 pairs (sic) of setae on femur II”. We suppose that Tseng (1985) based the identification on the genus description of Baker (1965) which states 2 setae on femur II. Actually, the genus description has 3 setae on femur II (André 1980). Tseng states “3 *ag* setae located anterior to the genital aperture”. The genus description of Baker (1965) mentions “4 *ag* setae anterior to and laterad from the genitalia”. We assume that the lateral *ag4* is therefore present but not mentioned. Most dorsal setal lengths are given. When compared to *P. ubiquitous*, the distinguishing character of *P. debilis* are the lanceolate, strongly tapering dorsal setae, the deviating lengths of setae *la* 26, *c1* 18, *c2* 26, *d1* 18 & *f2* 45 and (*p*ζ) 26 subequal and subequal with length of tarsus.

The second Tseng (1985) species is *P. perpusillus*. When compared to *P. ubiquitous*, the distinguishing characters of this species are the lanceolate, strongly tapering setae; the short dorsal setae; and the deviating lengths of setae *la* 14, *c1* 13, *c2* 13, *d1* 14, *f1* 17 & *f2*.25. This species resembles *P. brasiliensis* n. sp. as setal lengths fall within the range of those of *P. brasiliensis*, but the dorsal setae are conspicuously lanceolate.

When comparing with *P. ubiquitous* and *P. brasiliensis* n. sp., the dorsal setae of these species are also somehow lanceolate (Figure 15).

We conclude that redescription of both species is needed to confirm the difference in setal forms and the presence of *ag4*.

• Menon *et al.* (2007) described *P. oryzae*. Their description is more detailed, the mean and standard deviation of the dorsal setae are shown (not the range, we calculated the 99% confidence interval, see Table 5), and although leg chaetotaxy is not described, trochanter I appears without a seta on their drawing of leg I. We therefore believe that this species is indeed a *Pronematus*. When compared to *P. ubiquitous*, the distinguishing characters of this species are the short dorsal setae, *ro*, *la*, *c1*, *c2*, *d1*, *e1*, *f1* & *f2*.

The following species cannot be assured as belonging to *Pronematus* with certainty. We therefore add these five species to the list of *species inquirendae*. Redescriptions are needed:

• Ryke and Meyer (1960) described *P. sensillaris* in a minimalistic way having ωI 22, bo 22, prodorsal setae 15-19 and dorsal setae 12-20, tarsus I 23, tibia I 19 and *ft*"ζ 23. This long ωI and short *ft*"ζ are characteristics of male *Pronematus*. Study of the holotype is necessary to

confirm that it is indeed a female. We could not locate the types of this species.

- *Pronematus sextoni* (Baker 1968). Kaźmierski (1998) retained it on the list of *Pronematus* spp. Some authors have mentioned the presence in Türkiye (Soysal and Akyazi 2018; Çobanoğlu and Kaźmierski 1999), India and Africa according to Gupta and collaborators in Soysal and Akyazi (2018), Iran (Darbemamieh *et al.* 2021). Nevertheless, no redescrptions were made, and the description lacks any detail including leg setation as to assure it is a *Pronematus*. Many other species of Baker (1968) are now transferred to other genera and in our opinion redescription is needed to maintain it in the genus *Pronematus*.

- Gupta and Paul (1985) described *P. indiana* and *P. bengalensis*. The authors did not determine the leg chaetotaxy, but on the drawings of tarsus II of both species 3 dorsal setae appear which means that there would be 7 setae on tarsus II (4 distal setae unclear). Additionally, the terminal eupathidia on tarsus I of both species are very long, the prorals shorter, subequal. Finally, the habitat is birds' nests, while all other *Pronematus* spp. live on plants. This characteristic is found in e.g., *Pseudopronematus*, which live on fungi or in litter. Therefore, we doubt that these two species belong to the genus *Pronematus*.

- Gupta and Paul (1992) described *P. saularis*. The authors did not determine the leg chaetotaxy, but on the drawing of leg I, a seta appears on trochanter I, so this species cannot be a *Pronematus*.

Additionally, we studied the holotype of *P. tenuisetosus*, one of the *species inquirendae*, and concluded that it is a *Pseudopronematus*. This species will be treated in another publication.

We retain 8 confirmed *Pronematus* species: *P. debilis*, *P. karrooi*, *P. oryzae*, *P. perpusillus*, *P. rykei*, *P. juglandi*, *P. brasiliensis* **n. sp.** and *P. ubiquitous*. However, 4 species (*P. debilis*, *P. karrooi*, *P. perpusillus* and *P. rykei*) need full redescription including the range of the intraspecific variation. Possibly, only the combination of morphometric, genetic studies and crossbreeding experiments can define differences between species with certainty (Table 5).

Nevertheless, based on the available information, we developed the following key for separating the confirmed species. Some character measurements are close to each other, in case of doubt we refer to table 5 for all (known) measurements.

- 1 Dorsal setae lanceolate, acutely tapering from the widened proximal portion 2
- Dorsal setae not lanceolate, not acutely tapering from the widened proximal portion 3

- 2 (1) Dorsal setae longer, lengths of setae *la* 26, *c1* 18, *c2* 26, *dl* 18, *fl* 29 & *f2* 45; (*p*ζ) subequal 26 *P. debilis*
- Dorsal setae shorter, lengths of setae *la* 14, *c1* 13, *c2* 13, *dl* 14, *fl* 17 & *f2* 25 *P. perpusillus*

- 3 (1) Dorsal setae very short to short: *ro* < 20, *c1* < 17, *dl* < 19, *el* ≤ 21 4
- Dorsal setae longer: *ro* > 20, *c1* > 20, *dl* > 20, *el* ≥ 22 5

- 4 (3) Dorsal setae very short: *la* 10.3 ± 2.39 (9.3 - 11.2), *c1* 11.3 ± 2.26 (10.4-12.3), *c2* 12.3 ± 2.7 (11.2-13.4), *dl* 11.6 ± 1.84 (10.9-12.3), *el* 13.2 ± 2.65 (12.1-14.3) & *fl* 16.8 ± 3.72 (15.3-18.3) (mean ± st. dev (confidence interval 99%)) *P. oryzae*
- Dorsal setae short: *la* 15-23, *c1* 14-16, *c2* 15-18, *dl* 15-18, *el* 15-21 and *fl* 15-24. Palptarsal dorsal seta *d* forked (not always visible) *P. brasiliensis* **n. sp.**

- 5 (3) Prodorsal seta *la* 25.4, dorsal setae *c1* 27.1, Palptarsal dorsal seta *d* forked *P. rykei*
- Prodorsal seta *la* 22 or less, dorsal setae *c1* 25 or less, Palptarsal dorsal seta not forked or not known to be forked 6

- 6 (5) Dorsal setae *hl* 17, *f2* 25.3, bothridial seta (*bo*) 28.4 *P. karrooi*
- Dorsal seta *hl* 21 or longer, *f2* 30 or longer, bothridial seta (*bo*) 30 or longer 7

7 (6) Dorsal seta <i>hl</i> 25-27, ventral setae <i>lb</i> 20-22, <i>lc</i> 19-20, <i>4b</i> 13-18 and <i>ag4</i> 8-11.....	<i>P. juglandi</i>
— Dorsal seta <i>hl</i> 21-24, ventral setae <i>lb</i> 14-18, <i>lc</i> 13-18, <i>4b</i> 19-20 and <i>ag4</i> 13-14.....	<i>P. ubiquitous</i>

Discussion

Distribution

It is remarkable that *P. ubiquitous* has not been mentioned more frequently in acarological studies in Europe before. In fact, *P. ubiquitous* had only been found in Europe in Spain, Italy (including Sicily) (De Jong *et al.* 2014) while we found it in many European countries. Acarologists might have overlooked it, mostly focusing on mites from other families such as Phytoseiidae, which have been more intensively studied as biological control agents. This is supported by the frequent finding of iolinids including *P. ubiquitous* in e.g. Türkiye, Egypt and Iran, where many authors have studied or reviewed mite diversity in several crops or habitats (Abou-Awad 1979; Abou-Awad *et al.* 1999; Akyazi *et al.* 2016; Akyazi *et al.* 2017; Darbemamieh *et al.* 2021; Akyazi *et al.* 2022).

Pronematus ubiquitous has been found worldwide, but in most identifications no measurements were done. With this study, we confirm at least identifications of South Africa and Türkiye. Identifications in other countries can now be further confirmed. The Brazilian specimens that were used to describe *P. brasiliensis* **n. sp.** additional to the species studied in De Vis *et al.* (2006a) were not identified before as *P. ubiquitous* but as *Pronematus sp.* We suppose that in many other museum collections this might be the case. We hope that this can trigger taxonomist to study these specimens and so eventually discover new species.

However, the morphometric plasticity of the species as a result of climatic conditions, host plant or food remains unclear and may still hamper correct identification. Further studies using genetics and morphometrics are needed to elucidate this relation as to avoid the description of new species that in fact are not.

Habitat and feeding habits

Pronematus ubiquitous was described from citrus and we recovered the specimens for this study in California from a wide range of citrus varieties. However, the species lives or survives on a wide range of plants. In the middle east alone it was found on i.a. ornamentals (*Prunus laurocerasus*), vegetables (tomato), fruits trees or shrubs (persimmon-*Diospyros* spp., grape, pomegranate, date palm, mulberry, fig, apple), herbaceous plants (sugarcane, sorghum) or trees (oak) (Abou-Awad 1979; Abou-Awad *et al.* 1999; Akyazi *et al.* 2016; Akyazi *et al.* 2017; Darbemamieh *et al.* 2021; Akyazi *et al.* 2022). In this study, we found *P. ubiquitous* on wild plants such as *Rubus*, but it was much less frequently present than *H. anconai* which was present in about every sample of *Rubus* sp. More often, we found *P. ubiquitous* on cultivated ornamentals or fruit and vegetable crops. We therefore suppose that it can be found worldwide because it might have been traveling throughout the world on traded ornamental, vegetable or fruit plant material, where it might not always have been found by the plant inspection authorities because of its small size, particularly that of the eggs.

McGregor (1932) believed that *P. ubiquitous* fed on dead scale insects and their eggs, as they were commonly found in association with scale insects. Baker (1946) found it preying on *Eriophyes ficus* Cotte on figs in San Joaquin Valley California. It was found associated with fig trees in Egypt (Abou-Awad 1979) and Abou-Awad *et al.* (1999) defined its life cycle on eriophyid mites of the fig tree. Vervaet *et al.* (2022) showed its potential for the biocontrol of *A. lycopersici* and that it feeds also on *Typha* pollen. It needs plant material for reproduction. It probably feeds not only on the plant tissue or pollen of the plant on which it lives, but also

on aerial pollen that is deposited on plants during the pollen season. Recently, it has been hypothesized that it might also feed on fungi like powdery mildew (Pijnakker *et al.* 2022b). In contrast, the specimens used in De Vis *et al.* (2006a), in this study identified and described as *P. brasiliensis* n. sp., do not need plant material for reproduction. They could be reared easily on a rearing unit with a linoleum basis, bordered with water-soaked cotton and with just pollen as food source and cotton under a cover glass as oviposition substrate. Although it was found on tomato, it is not clear that these plants were infested with tomato russet mite. De Vis *et al.* (2006a) did not test the species as biological control agent of tomato russet mite, however, it did not reproduce on the eriophyid *Calacarus heveae*.

Taxonomy

Unguinals and seta homologous to *s*. During our studies, we discovered two peculiarities with respect to the distal setation on tarsus I: First, the two unguinal setae are forked from the second third of their length where the two laciniae are about double as long as the common base (Figure 2, 3 & 10). The position of both unguinal setae is proximal and a bit lateral to two prorals $p'\zeta$ and $p''\zeta$. Additionally, we discovered that in between $p'\zeta$ and $p''\zeta$ there is a small smooth seta homologous to *s* of about 2 μm (Evans 1992). It seems that this seta has been overlooked previously or confused with one of the unguinals. We suppose that it might be general in Pronematinae as we could find this seta also on *H. anconai* we collected during this study, but also on other Pronematinae species. We also assume that this seta is erroneously named *u'* in Figure 1a & 1b in Knop and Hoy (1983a), as the position of *u'* is ventral and proximal to $p'\zeta$ and the depicted *u'* is in between the two prorals.

Which characters are taxonomically reliable. The absence of shields or sclerotized structures reduces the options for morphological identification. Setae are inserted on the integument so distances between them are subject of variation due to mounting, the size of the specimen or its physiological status, which makes these characters unreliable. The organism's small size means that the smallest setae are also difficult to measure correctly because they are often pushed up, bent upwards or downwards in the mounting medium and are not visible in one plane, and because of their small size the error increases. This is also the case for lateral setae *ex* and *c2*, that are inserted on the lateral side of the dorsum and mostly are not visible in 1 plane. Equally for the tarsus I setae. Legs are mostly not pressed between slide and cover glass because the body impedes this. This can in part explain the lower loading of these setae in the PCA (Figure 8A). Using less mounting medium can overcome this problem and might be important when mounting specimens for description. The dorsal and coxal setae had the highest loading in the PCA and therefore appear to be the most reliable characters (Figure 8A).

It appears that also ωI , frequently used to distinguish between pronematid species, is very variable within *Pronematus*. Californian (USA) specimens have ωI varying between 5 and 15, with the mean of 7.1 μm . In one specimen originating from a SFEL, we found a ωI of 15 μm on one leg and the "normal" length on the other leg, whereas in another specimen both legs had ωI 15 μm .

It might also be that characters that were not studied or might have been overlooked are of more importance than first thought. For instance, the dorsal seta *d* of the palp tarsus is forked in *P. rykei* and *P. brasiliensis* n. sp.. This occurred also with the taxonomy of *Kampimodromus* spp. (Acari: Phytoseiidae) where the number of solenostomes and the presence of a tooth are more important than setal lengths (Tixier *et al.* 2008). Similarly, only the serration of the macroseta on the basitarsus allows the differentiation between *P. persimilis* and *P. macropilis* (Acari: Phytoseiidae) (Okassa *et al.* 2010).

DNA barcoding or morphological identification. DNA barcoding could circumvent above-mentioned limitations and the time-consuming morphological determinations. A limitation of the barcoding is, however, the small size of the Pronematinae. With one well developed adult female, DNA extraction is not always successful and when preserved in alcohol, at least 3 to 4 females are necessary for one analysis. Identification of a single wild specimen is therefore not

trivial and SFEL cultures are needed, which is also time-consuming. Further optimising of the method might make it possible in the future with only one female. The method of Tixier *et al.* (2010), where after extraction of the DNA from a specimen, the carcass is recovered for slide mounting, was not tested but might not be applicable to the very small and fragile Pronematinæ that lack shields.

The COI gene sequence is used extensively for arthropod identification (Hebert *et al.* 2003; Hebert *et al.* 2004; Tixier *et al.* 2008; Boehme *et al.* 2012; Li *et al.* 2012; Ovalle *et al.* 2014; Doña *et al.* 2015; Schäffer *et al.* 2019; Małkol *et al.* 2019). Although the 25 *P. ubiquitous* sequences all formed a cohesive cluster, two closely related subgroups were apparent. One group clustered the European strains (Italy, France, Belgium, and The Netherlands) and one comprised the non-European strains (USA and Morocco). All European samples are almost identical both morphologically as well as molecularly, except for samples 50.1 and 50.2 that separate in the tree from the other European specimens. This could, however, not be seen in the morphological characteristics of the SFEL specimens which fall within the range of the other French specimens. A possible explanation for the high similarity of the sequences originating from one locality is the limited genetic basis as the laboratory cultures were started with few females. Nevertheless, the variability of the morphological characters of specimens coming from the SFEL's was similar to that of wild specimens that were found on 8 localities in Belgium or 4 localities in France, indicating that all these specimens belong to the same species.

We do not separate the European specimens as a distinct species because of the overlap in setal lengths with specimens from other origins, visible in Figure 8B and Table 2. Reassuringly, the samples differentiate in a similar way when comparing the classical morphological method and the PCA (Figure 8B) with the molecular method and the phylogenetic tree (Figure 7). Whether the observed genetic and morphological differences between European and non-European specimens are due to geographic separation is not proven. Therefore, much more independent samples, spread throughout each country or region should be analysed.

We suppose that the barcoding can differentiate between genera as *H. anconai* is clearly separated from *P. ubiquitous* in the phylogenetic tree (Figure 7). Some intraspecific variation was found between the *P. ubiquitous* samples but the interspecific variation is greater than the intraspecific variation. André and Fain (2000) studied the phylogeny in the Tydeoidea and considered that species with less setae arose more recently. In this reasoning *P. ubiquitous* might have arisen more recently than *H. anconai* because of the loss of setae on the legs and the loss of *ps2*. Although we see this not reflected in the phylogenetic tree, it does reflect the gradually lower phylogenetic relationship of *H. anconai* and *P. ubiquitous* with *Microtydeus* sp., *Tydeus* sp., *T. urticae*, *P. citri*, *P. ulmi*, and the mesostigmatid mite *V. destructor*.

To date, no COI sequences were available for *P. ubiquitous* and by extension for Iolinidae and the 6 unique COI sequences reported here constitute the first barcode for this species. However, DNA sequencing of more species, preferably with more than one marker gene is required to elucidate relationships. Studies have shown that when COI is combined with other marker genes such as 28r-RNA, clade structure can change (Duarte *et al.* 2023). This underscores the importance of additional research, integrating barcoding with morphological identification as to make the species delimitation more reliable (Puillandre *et al.*, 2012). Preferably such research should be performed using confirmed and distinct species of the same genus, subpopulations of the same species and/or combined with crossbreeding studies to identify those subpopulations (Anderson and Trueman 2000; Tixier *et al.* 2008). In this study it was, however, not possible to study more than one *Pronematus* species molecularly.

In line with Bergmann's rule, we found that latitude seems to be positively correlated with *el* length (Figure 9). This intraspecific variation as affected by the latitude of the collection location could explain some of the observed differences. Hence, when discussing differences between sizes or lengths of the same species, this temperature-size rule needs to be considered.

Other species

Unlike *P. ubiquitous*, all the other *Pronematus* species have only been found in specific regions or crops. *P. brasiliensis* n. sp. is described from different regions in Brazil, from the northeast to the southeast and on different plants and crops. *P. juglandi* has only been collected in Iran, on walnut trees, *Juglans regia*, infested by leaf gall mite, *A. tristriatus* Nalepa, which causes a dense erinea. *P. rykei* was described from cotton in Mozambique and *P. karrooi* from *Acacia karroo* in South Africa. *P. oryzae* was only described from rice in New Delhi, India and two species were described from Taiwan: *P. debilis* from two different places on papaya and guajava, and *P. perpusillus*, from an unidentified plant.

Differences between species are sometimes very small. For instance, only the shape of the setae of *P. debilis* and *P. perpusillus* distinguishes them now from some other species. Together with *P. karrooi* and *P. rykei* they need full redescription. This might lead to new synonymies. On the other hand, the redescription of *P. rykei* made clear that the Iranian specimens of that species do not match the original description, leading to a new species, *P. juglandi*. It shows that effort should be made to redescribe the species that were not fully described, based on the type specimens or if not available, based on topotypes, as we did for *P. ubiquitous*. We may not exclude the *species inquirendae* from that effort, as the study of the holotype of *P. tenuisetosus* showed that it is a *Pseudopronematus* species, which will be treated in another publication. The search for and loan or exchange to experts of types among experts is essential in this process.

Acknowledgements

This research was mostly done during the first two authors' free time. In part, it was supported by the projects DUCATO, financed by LAVA, the Flemish Vegetable Auctions Association, and BALTO, "Beheersing van *Aculops lycopersici* in tomaat", funded by Flanders Innovation & Entrepreneurship (grant HBC.2017.0829). We are grateful to all the collectors of materials, i.a. Johan Foqué & Helena Bartier, Robin Van Havermaet, Felix Wäckers and many collaborators of the PSKW that brought samples. We are greatly indebted to Gilberto JG de Moraes who kindly made available slides of Brazilian specimens of the museum collection at MZQL, ESALQ, USP, Piracicaba, Brazil. Thanks is also due to Dr Rana Akyazi, of the Ordu University Türkiye for permission to include her *P. ubiquitous* material. Thanks go to Negin Ebrahimi, Johan Witters and Dieter Slos of ILVO for making available the DIC-microscope at Ilvo and help us making pictures.

Authors' contributions

Edward A. Ueckermann made the redescription and drawings of *P. ubiquitous* and measured the specimens of the type locality (first collection), South Africa and Türkiye; he also measured and redescribed *P. rykei* and *P. karrooi*, but he was most importantly a rock in the ever increasing and turbulent *Pronematus* waves; Raf M.J. De Vis coordinated the survey, initiated laboratory populations, made the redescription, took pictures, and measured all the other specimens; he also made the review and the key; Lore Vervaeke set up SFEL and performed the molecular study; Eva Reybroeck coordinated the survey and made a lot of slides, Wendy Van Lommel participated in the survey; Thomas Van Leeuwen and Patrick De Clercq supervised the work of Lore Vervaeke; Sandipa Gautham and Yuling Ouyang collected (first and second) and reared the specimens and SFEL of the type locality, Dominiek Vangansbeke participated in the survey, reared specimens at Biobest and made the PCA analysis.

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