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Molecular Phylogenetics and Light Microscopy Reveal "True" and "False" Calacarines and Novel Genital Structures in Gall Mites (Acariformes, Eriophyoidea)

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Abstract: Gall mites (Eriophyoidea) are cosmopolitan microscopic phytoparasites that often transmit viruses and induce gallogenesis. The tribe Calacarini is diagnosed by a set of plesiomorphic and homoplastic traits, including elimination of setae *sc* shared with other lineages of Eriophyoidea. We reviewed data on the generic diversity of calacarines, revised the concept of the type genus *Calacarus* Keifer 1940, and proposed three zones (MZ, SMZ, LZ) in the prodorsal shields of calacarines to simplify descriptions of their shield patterns. We describe three new calacarine species (*Calacarus baviensis* **n. sp.**, *C. burchelliae* **n. sp.**, and *Viginticus searsiae* **n. sp.**) from indigenous dicotyledonous trees from South Africa and Vietnam and report on new findings of *Paracalacarus podocarpi* Keifer in Brazil, *Jiangsuacarus* sp. in the USA, and *Calacarus pusillus* Pye in Latvia and Russia. The latter represents the new most northern locality of Calacarini. Reinvestigating the type species of *Jaranasia* Chandrapatya & Boczek 2000 revealed that absence of setae *l*" II is the only character separating it from *Jiangsuacarus* Xue 2009. We proposed two new combinations: *Jiangsuacarus sesleriae* (Skoracka 2004) **n. comb.** (transferred from *Jaranasia*) and *Procalacarus mussaendae* (Keifer 1977) **n. comb.** (transferred from *Calacarus*). Partial sequences of *Cox1* and *28S* genes were obtained for six calacarines, some of them originating from old ethanol material kept at room temperature. Molecular phylogenetics revealed a stable cluster of "true" calacarine sequences comprising *Calacarus*, *Jaranasia*, *Latitudo*, and *Viginticus* and a polyphyletic group of erroneous sequences assigned to Calacarini in GenBank. All investigated females of calacarines have a pair of genital tubules associated with the vestibulum and hypothesized to participate in fertilization. This finding may contribute to resolving the question on how the fusion of gametes happens in gall mites.

Keywords: Acari; phytophagous mite; phytoparasite diversity; *Calacarus*; *Cox1*; *28S*; endemic; erroneous sequences; female genitalia; arthropod structure; reproductive system

Citation: Chetverikov, P.E.; Craemer, C.; Gankevich, V.D.; Le, N.T.T.; Nguyen, V.D.; Trinh, H.X.; Amrine, J. Molecular Phylogenetics and Light Microscopy Reveal "True" and "False" Calacarines and Novel Genital Structures in Gall Mites (Acariformes, Eriophyoidea). *Forests* **2024**, *15*, 329. https://doi.org/10.3390/f15020329

Academic Editor: Bruno Massa

Received: 5 January 2024
Revised: 5 February 2024
Accepted: 6 February 2024
Published: 8 February 2024

1. Introduction

Eriophyoids or gall mites (Acariformes, Eriophyoidea) are microscopic phytoparasites permanently associated with higher vascular plants [1,2]. Some are capable of vectoring plant viruses, causing toxemias, and inducing growth abnormalities including witches' brooms and variously shaped leaf, bud, bark, and flower galls [3–6]. Due to serious pathological effects on plant tissues caused by activities of many eriophyoid mites such as discoloration, bronzing, necrosis, suppression of photosynthesis, and destruction of

vegetative and reproductive buds, many are economically important pests necessitating regulations and control in international trade and agriculture [3,7,8]. The majority have little or no impact on their host plants.

Eriophyoid mites are cosmopolitan and present in all areas where their host plants grow [9]. Because of their high host-specificity [10], species of gall mites can be expected to mirror distributions of their hosts, and if a host is introduced to a new area without adequate quarantine measures the simultaneous introduction of its eriophyoid associates is quite likely.

The genus *Calacarus* Keifer 1940 is a large economically important taxon of gall mites. It includes such well-known pest species as *C. brionesae* Keifer 1963, *C. carinatus* (Green 1890), *C. citrifolii* Keifer 1955, and *C. coffeae* Keifer 1960, seriously damaging papaya, tea, citrus, orange, coffee, and other crops [11]. *Calacarus* spp. (as well as the whole tribe Calacarini) are largely associated with various tropical hosts and described mainly in Asia (mostly China, Thailand, and India), South Africa, the USA, and Brazil, and only one species, *Calacarus pusillus* Pye 2012, had been previously recorded in Europe (in Great Britain) [12]. Several other eriophyoid lineages, e.g., members of Mackiellini, Aberoptinae, and Diptilomiopinae, are known to dominate in tropical and subtropical areas where they are mainly recorded from various trees and shrubs in native forests, as well as agroecosystems and parks [9,13,14].

In a recent study, Li et al. [15] investigated the global distribution and diversity of eriophyoid mites and suggested that species richness and endemism of eriophyoids peak in temperate regions, in contrast to the patterns of plants and some other organisms. However, an immediate follow-up critique paper [16] provided strong evidence that Li et al. [15] (a) referred to previous literature incorrectly, (b) based their analyses on an insufficient, geographically biased dataset, and (c) provided controversial interpretations contradicting their own results. The major message coming from this critique paper [16] conforms with the traditional view of eriophyoid distribution: (a) extrapolating from extremely high plant diversity in tropical environments, Eriophyoidea are likely to be more diverse in the tropics than in other regions worldwide; (b) the apparent skewed distribution results from the majority of past research being conducted in temperate regions [17].

According to our estimates, the tribe Calacarini currently includes 19 genera and ~80 species, most of which (~65%, about 50 spp.) belong to the type genus *Calacarus* Keifer 1940. The majority of calacarines produce wax and are purple or violet-black, contrary to colors much more common for Eriophyoidea: white, red, orange, or yellowish colorization. The nature and function of the chemical compounds responsible for color in gall mites is unknown. Descriptions of many calacarines show they are morphologically very similar, sometimes creating the impression that some species were described several times under different names (thus, are synonyms). This situation is quite common in Eriophyoidea [17]. However, like various species of other eriophyoid genera, e.g., *Aceria* and *Abacarus* from monocots [18,19], *Phytoptus* and *Phyllocoptes* from dicots [6,20,21], and *Trisetacus* from conifers [22,23], some calacarines may represent complexes of cryptic species, explaining their morphological similarity. This evolutionary trend has been shown to be highly common in Eriophyoidea [24]. For instance, *Calacarus citrifolii*, a pestiferous species associated with citrus but also infesting an untypically wide range of phylogenetically unrelated host plants, is suspected to be a complex of sibling species (J.A., C.C., and P.C., unpublished observation).

Molecular-based methods provide an effective tool for resolving taxonomic and phylogenetic problems in various fields of acarology. Since the end of the twentieth century, comparison of sequences of marker genes has become a routine procedure widely used in eriophyoid studies [25]. Intensive accumulation of gene sequences of Eriophyoidea in public databases (e.g., GenBank) happened in the last decades due to prolific molecular studies on Eriophyoidea in China, Poland, Italy, Serbia, the USA, and Russia. This significantly expands our knowledge on genetic diversity of gall mites and facilitates our ability to test their conspecificity [6,20,26,27]. Additionally, various species delimitation

methods, as well as Blast and tree-based approaches, facilitate testing species boundaries, validate morphological identification, and detect erroneous sequences of gall mites, like those deposited in public databases [28–30]. The latter is especially important for obtaining correct datasets for molecular phylogenetic analyses and avoiding misleading conclusions.

In this paper, we describe three new species of two calacarine genera, *Calacarus* Keifer 1940 and *Viginticus* Duarte & Navia 2020, from indigenous dicotyledonous trees from South Africa and Vietnam and give new records of calacarines from Brazil, the USA, Latvia, and Russia (Figure 1). We also obtained partial sequences of two genes (*Cox1* and *28S*) of the calacarine specimens from our material and performed extensive Blast searches to assess taxonomic identity of our sequences and all sequences of calacarines that are currently present in GenBank. Finally, we investigated the phylogenetic position of the new calacarine taxa in Eriophyoidea and assessed the integrity of the tribe Calacarini based on the results of the two single-gene analyses. Additionally, we describe and discuss the possible function of genital tubules, the new structures detected in all our slide-mounted calacarines.

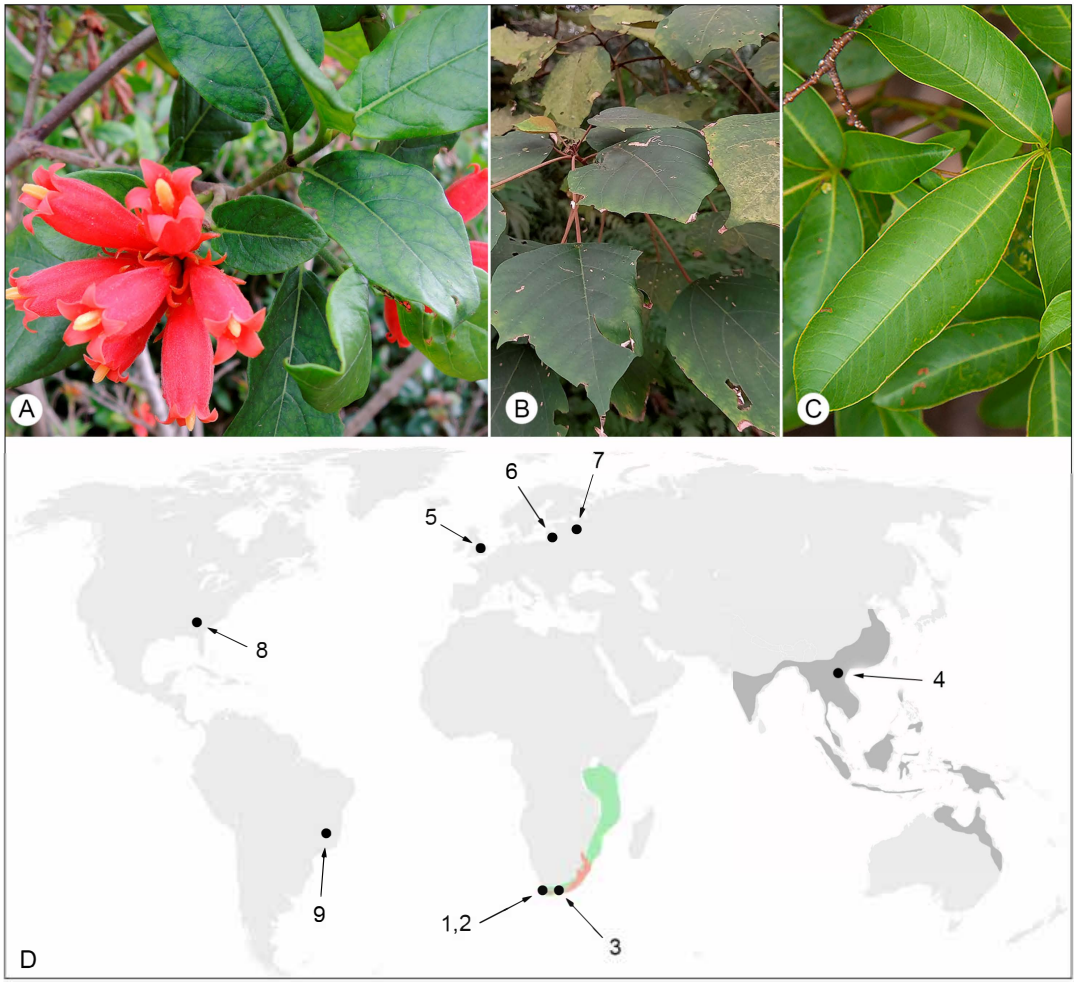

Figure 1. Host plants (**A**–**C**) and collecting sites (**D**) of five calacarine species. (**A**)—*Burchellia bubalina* (South Africa), (**B**)—*Mallotus paniculatus* (Vietnam), (**C**)—*Searsia chirindensis* (South Africa). (**D**)—Type localities of *Viginticus searsiae* **n. sp.** (1) and *Calacarus burchelliae* **n. sp.** (2) in South Africa, *C. baviensis* **n. sp.** (4) in Vietnam, *C. pusillus* (5) in Britain, locality of additional population of *C. burchelliae* **n. sp.** (3) and new findings of *C. pusillus* in Latvia (6) and northwestern Russia (7), *Jiangsuacarus* sp. in the USA (8), and *Paracalacarus podocarpi* in Brazil (9). Photos—P.E. Chetverikov. Approximate distribution areas of host plants *B. bubalina*, *M. paniculatus*, and *S. chirindensis* are shown with pink, dark gray, and green (respectively) according to data from [31–35].

2. Materials and Methods

Collection and morphological measurements. The leaves and branches of six plant species were sampled in South Africa, Vietnam, Latvia, the USA, Brazil, and Russia in 2010–2023 (Table 1) and examined under a stereo microscope. The mites were collected using a minuten pin, slide-mounted in modified Berlese medium with iodine [36] and cleared on a heating block at 90 °C for 3–5 h. Some mites were stored in Eppendorf tubes filled with 96% ethanol and kept in a refrigerator (−25 °C) for DNA extraction or kept alive on leaves in a refrigerator (+3 °C) for further examination under low temperature scanning electron microscopy (LT-SEM).

Table 1. Collecting data and GenBank accession numbers for six calacarine mite species.

Mites Species	Collecting Data	GB Accession Numbers	
		Cox1	D1D2 28S
Calacarus baviensis **n. sp.**	VIETNAM: Ba Vi National Park, 21°05′03.4″ N 105°23′00.9″ E 17 February 2023, from upper leaf surface of *Mallotus paniculatus* (Lam.) Müll. Arg. (Euphorbiaceae), coll. P.E. Chetverikov, L.T.T. Nhung, N.D. Viet	OR756238	OR789152
Calacarus burchelliae **n. sp.**	SOUTH AFRICA (type population): Cape Town, near Kirstenbosch National Botanical Gardens, 33°59′09.4″ S 18°26′01.7″ E, 12 November 2016, from lower leaf surfaces of *Burchellia bubalina* (L.f.) Sims (Rubiaceae), coll. P.E. Chetverikov, C. Craemer, S. Neser	OR756236	OR789151
	SOUTH AFRICA (additional population): mountain road from Avontuur to Knysna near bridge over the Diep River, 33°51′38.3″ S 23°10′23.0″ E, 6 November 2016, same host and relation to host, coll. P.E. Chetverikov, C. Craemer, S. Neser	OR756237	100% identity with OR789151
Calacarus pusillus Pye 2012	RUSSIA: Pskov Prov., Loknya Dstr., near vill. Sosnovo, swampy pine forest, 57°00′29.9″ N 30°28′45.5″ E, 12 July 2010, from leaves of *Calluna vulgaris* (L.) Hill (Ericaceae), coll. P.E. Chetverikov	-	-
	LATVIA: Ventspils Prov., Uzava Dstr., near Ziras, pine forest on the coast of the Baltic sea, 57°09′41.7″ N 21°25′02.5″ E, 26 July 2019, from leaves of *Calluna vulgaris* (L.) Hill (Ericaceae), coll. P.E. Chetverikov	OR756239	OR789154
Jiangsuacarus sp.	USA: Dekalb Co, GA, South River at GA. RT. 155 and Panaloa RD, 27 May 2019, from leaf underside of *Arundinaria gigantea* (Walter) Muhl. (Poaceae), purple, coll. J.W. Amrine	OR756240	OR789155
Paracalacarus podocarpi Keifer 1962	BRAZIL: Minas Gerais Belo Horizonte, Rua Expedicionário Paulo de Oliveira, 19°51′51.0588″ S, 43°58′43.1322″ W, 07 Apr 2015, from dorsal surface of young leaves of ornamental *Podocarpus* sp. (Podocarpaceae), yellowish color, coll. P.B. Klimov	-	OR789156
Viginticus searsiae **n. sp.**	SOUTH AFRICA: Cape Town, near Kirstenbosch National Botanical Gardens, 33°59′19.9″ S 18°25′56.1″ E, 12 November 2016, along veins on upper leaf surface of young leaves of *Searsia chirindensis* (Baker f.) Moffett (Anacardiaceae), coll. P.E. Chetverikov, C. Craemer, S. Neser	OR756235	OR789153

External morphology of the slide-mounted specimens was studied using conventional light microscopy (LM) using a Leica DM2500 and photographed with a ToupCam UCMOS09000KPB digital camera (Hangzhou ToupTek Photonics Co., Hangzhou, China). Morphological descriptions of all new species were based on phase contrast (PC) and differential interference contrast (DIC) LM observations and supplemented (for one species) with LT-SEM data obtained using a previously described methodology [14].

All measurements were obtained using ToupTek ToupView software (http://www.touptek.com/download/showdownload.php?lang=en&id=33, accessed on 5 February 2024). They are provided in the descriptions in micrometers (μm) and are lengths except when stated otherwise. The measurements of females are based on the holotype, whereas the ranges (in brackets) are based on measurements of the paratypes and holotype. In the descriptions of all other instars, only ranges are provided. Terminology of eriophyoid morphology and classification of Eriophyoidea follow [2,17], respectively. For description of the prodorsal shield ornamentation of the new Calacarini, we divided the shield in three zones (MZ, LZ, and SMZ) as shown in Figure 2A and referred to these zones in the textual species descriptions. Drawings of mites were sketched by pencil using a video projector [37], scanned, and finalized in Adobe Illustrator CC 2014 (Adobe Systems, San Jose, CA, USA) using a Wacom Intuos S CTL-4100K-N (Wacom Co., Ltd., Kazo, Saitama, Japan) graphics tablet.

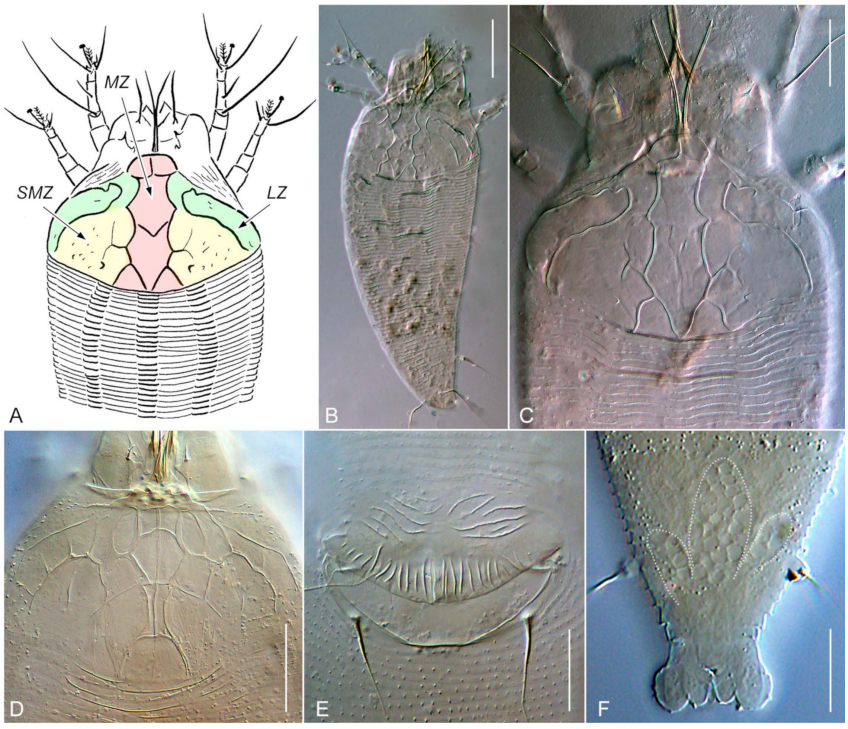

Figure 2. Prodorsal shields (**A**,**C**,**D**), dorsal view of female (**B**), external genitalia (**E**), and partially dissolved anal glands (outlined) (**F**) of *Calacarus pusillus* Pye 2012 from Great Britain ((**A**), redrawn from [12]) and Russia ((**B**,**C**)—DIC LM microphotographs) and *Paracalacarus podocarpi* Keifer 1962 from Brazil ((**D**–**F**)—DIC LM). Three zones of prodorsal shield are indicated in (**A**): medial zone (MZ, pink) flanked by admedian lines, lateral zone (LZ, green) along anterior margin of prodorsal shield, and submedian zone (SMZ, yellow) situated between MZ and LZ. Scale bars: (**B**)—30 μm, (**C**)—15 μm, (**D**)—20 μm, (**E**,**F**)—10 μm.

In order to review the morphological characters discriminating supraspecific taxa of Calacarini, we (a) screened original descriptions of calacarines from our libraries and the internet, (b) compiled Table 2, showing morphological "portraits" of all current calacarine genera, and (c) compared it with the current classification of the tribe Calacarini [17] and with the results of the molecular phylogenetic analyses (reported in the Section 3.2) focused on the phylogenetic position of the new calacarine taxa.

Table 2. Main morphological characters distinguishing genera of Calacarini. Notations: "+" present, "−" absent, "?" not clear from original description.

	Calacarus Keifer 1940	*Hornophyes* Mohanasundaram 1994	*Jaranasia* Chandrapatya & Boczek 2000	*Jiangsuacarus* Xue et al. 2009	*Jutarus* Boczek & Chandrapatya 1989	*Kolacarus* Boczek 1998	*Latitudo* Huang 2001	*Lanyuii* Wang & Huang 2012	*Liroella* Amrine 1996	*Neopentamerus* Kuang 1993	*Paracalacarus* Keifer 1962	*Paraphetelaburus* Xue et al. 2007	*Parinarus* Chandrapatya & Boczek 2000	*Phaulacus* Keifer 1961	*Procalacarus* Mohanasundaram 1983	*Quadratum* Huang & Wang 2004	*Rapinarus* Chandrapatya et al. 2016	*Spinacarus* Xue et al. 2009	*Taijutarus* Huang & Wang 2004	*Vigintiicus* Duarte & Navia 2020
Tubercles of *sc*	+/−	+	−	−	−	−	−	−	−	−	−	+	−	−	+	−	−	−	−	+
Tibial setae *l′* I	+	+	+	+	+	−	−	+	+	+	+	+	+	+	−	+	+	+	+	+
Genual setae *l″* II	−	+	?−	+	−	+	−	+	−	−	−	−	+	−	−	−	−	+	+	+
Femoral setae *bv* I	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+
Femoral setae *bv* II	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+
Opisthosomal setae *c2*	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	−	+
Opisthosomal setae *d*	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Opisthosomal setae *e*	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
Lateral projections of opisthosomal annuli	−	+	−	−	−	−	−	?+	−	−	−	−	−	+	−	−	−	+	−	−
Dorsoventral differentiation of opisthosomal annuli	+	+	−	+	+	+	+	+	?+	−	−	−	+	+	+	+	+	+	+	−
Median opisthosomal furrow	−	−	−	−	?+	−	−	−	+	−	−	−	+	−	−	?−	+	?+	−	−
Median opisthosomal ridge	+	−	−	−	−	−	−	−	−	+	+	+	−	−	+	−	−	−	−	−/+
Dorsal opisthosoma evenly rounded	−	+	+	+	−	+	−	−	−	−	−	−	−	−	−	?+	−	−	+	+
Three or five distinct longitudinal opisthosomal ridges	+	−	−	−	−	−	−	+	−	+	+	+	−	−	+	−	−	−	−	−
Tarsal solenidia ω with large spherical knob	+	+	−	−	+	−	+	−	?+	+	−	−	−	−	+	?−	−	−	?−	+

Molecular phylogenetics. For molecular studies, we obtained partial sequences of two genes (*Cox1* and *28S*) of six calacarines (Table 2) using our previously described methodology and protocols for DNA extraction, PCR, and sequencing [30,38]. The new sequences of calacarines were blasted against sequences of Eriophyoidea from GenBank. We also performed Blast searches for all *Cox1* and *28S* sequences of calacarines that are currently present in Genbank (23 October 2023). After that, two sequence datasets (*Cox1* and *28S*) were made for molecular phylogenetic analyses.

For creating the *Cox1* dataset, we blasted the sequence OR756239 of *Calacarus pusillus* against Eriophyoidea, filtered 3183 resulted sequences of >30% coverage, removed duplicates using sRNA-toolbox [39], added our new *Cox1* sequences of calacarines (Table 2), aligned the sequences in MAFFT [40,41] with defaults adjustments, and checked the obtained alignment for the absence of stop-codons. Thereafter, we removed (a) all sequences of Phytoptidae s.str., Pentasetacidae, and Nalepellidae except *Nalepella*, *Setoptus*, and *Boczekella*, which were used as outgroups, (b) all sequences containing indels and more than 8 degenerate nucleotides (R, Y, N, etc.), and (c) those which were notably shorter from the 3′ and/or 5′ end. The final *Cox1* alignment contained 580 sequences and 1155 nucleotide positions (385 amino acid positions).

For creating the *28S* dataset, we combined all D1D2 *28S* sequences of Calacarini from GenBank with our sequences (Table 2) in a single fasta file (contained 25 sequences), blasted it against Eriophyidae s.l., and filtered by coverage >30%, added all relevant *28S* sequences of *Nalepella*, *Setoptus*, and *Boczekella* (outgroups) from GenBank, and aligned all sequences in MAFFT using the E-INS-i iterative refinement method. The final *28S* alignment contained 225 sequences and 1961 nucleotide positions.

Two maximum likelihood (ML) analyses were performed in IQ-TREE 2.1.2 [42] using the Galaxy platform [43] through the Galaxy Eu server (https://usegalaxy.eu/) (accessed on 23 November 2023). Models LG+F+R8/GTR+F+R7 for *Cox1* (translated into amino acids)/*28S* gene evolution were selected using ModelFinder [44], as implemented in IQ-tree 2 based on the Akaike information criterion. Branch support values were generated from the Ultrafast bootstrap approximation [45] with 10,000 bootstrap alignments, 10,000 maximum iterations, and a minimum correlation coefficient of 0.99. Values of a single branch test (SH-like approximate likelihood ratio test, SH-aLRT) with 10,000 replicates and Ultrafast bootstrap support (UFBS) were labeled on the ML trees.

3. Results

3.1. Taxonomy

Family Eriophyidae Nalepa 1898

Subfamily Phyllocoptinae Nalepa 1892

Tribe Calacarini Amrine & Stasny 1994

Diagnosis. Prodorsal shield setae *sc* absent, tubercles of *sc* present or absent, short-form oral stylet, female external genitalia not appressed to coxisterna II, female internal genitalia as usually shaped in most Eriophyidae (except Cecidophyinae), empodia simple, all leg segments present and unmodified.

Remarks on diagnosis of Calacarini. This tribe was established to incorporate Phyllocoptinae with non-divided empodia that lack setae *sc* [17]. Outside Phyllocoptinae, reduction of *sc* convergently happens in one genus of Nalepellidae (in *Boczekella*), in four genera of Phytoptidae s. str. (in *Neopropilus*, *Neoprothrix*, *Palmiphytoptus* (although in literature this genus belongs to Phytoptidae, it may actually be a member of Eriophyidae [17]), and *Propilus*), and in two groups of genera in the subfamilies Nothopodinae (in the tribe Nothopodini) and Cecidophyinae (in the tribe Cecidophyini). A similar reduction trend is also obvious in one of the two subfamilies of Diptilomiopidae: it is common in Diptilomiopinae, but rare in Rhyncaphytopinae (e.g., *sc* are diminutive in *Areekulus* and absent in *Sakthirhynchus*).

Besides a homoplastic reduction of *sc*, the current concept of Calacarini implies all members of this tribe possess (a) commonly shaped female internal genitalia with a well-developed longitudinal bridge and anterior genital apodeme situated in the frontal plane (contrary to cecidophyines and novophytoptines, which have this apodeme apomorphically situated in the transverse plane) and (b) commonly shaped legs with normal segmentation and position of tarsal appendages (contrary to nothopodines showing advanced conditions with reduced or completely lost tibiae and often displaced tarsal empodia and solenidia). Therefore, all characters defining Calacarini are either homoplasies or plesiomorphies. Moreover, when conditions of (a) and (b) are unknown for a certain calacarine taxon (e.g., not mentioned in the original description), it is impossible to conclude whether it is indeed a true member of Calacarini or whether it belongs to Cecidophyini or Nothopodini. In such cases, DNA-based methods may be helpful if fresh material for DNA extraction and PCR becomes available.

Genera included (*n* = 20) (Table 2): *Calacarus* Keifer 1940, *Hornophyes* Mohanasundaram 1994, *Jaranasia* Chandrapatya & Boczek 2000, *Jiangsuacarus* Xue et al. 2009, *Jutarus* Boczek & Chandrapatya 1989, *Kolacarus* Boczek 1998, *Latitudo* Huang 2001, *Lanyuii* Wang & Huang 2012, *Liroella* Amrine 1996, *Neopentamerus* Kuang 1993, *Paracalacarus* Keifer 1962, *Paraphetehaburus* Xue et al. 2007, *Parinarus* Chandrapatya & Boczek 2000, *Phaulacus* Keifer 1961, *Procalacarus* Mohanasundaram 1983, *Quadratum* Huang & Wang 2004, *Rapinarus* Chandrapatya et al. 2016, *Spinacarus* Xue et al. 2009, *Taijutarus* Huang & Wang 2004, *Viginticus* Duarte & Navia 2020.

Remarks. The set of morphological characters discriminating calacarine species includes the following: presence/absence of tubercles of *sc*, leg setae l' I, l'' II, *bv* I and II, opisthosomal setae *c2*, *d* and *e*, large spherical knob of ω, and topography of opisthosomal annuli (dorsoventral differentiation, ridges, and furrows) (Table 2). Differences in chaetom

are more reliable for taxonomic purposes because the presence or absence of a seta is a discrete character, whereas the topography of opisthosoma is a descriptive and often continuous trait that may be variable and/or distorted by artefacts caused by clearing and slide-mounting. For instance, in the monotypic genus *Viginticus*, "dorsum is evenly rounded, but some specimens present a slight ridge anteriorly" [46].

Descriptions of some calacarine taxa are inadequate mainly because they include inadequate drawings and incomplete or uncertain textual descriptions. Therefore, it is hard to determine whether some of other genera are synonyms or belong to another suprageneric taxon, e.g., some *Jutarus* and *Kolacarus* from Asia may belong to *Cecidophyinae* according to their external morphology depicted in taxonomic descriptions; however, the decisive data on internal genitalia is not available [47].

Monotypic genera *Lanyuii*, *Taijutarus*, and *Quadratum* are illustrated very poorly; however, they are easily recognizable due to the absence of *c2* (in *Lanyuii* and *Taijutarus*) or *e* (in *Quadratum*) [48,49]. Type material is unavailable; synonymy of *Lanyuii* and *Taijutarus* is highly probable.

Description of *Phaulacus lanyuensis* Huang 2001 states that setae *l*'' II are absent, but they are clearly present in Figure 3C (lateral mite) [48]. Additionally, the habitus of this species very closely resembles that of some *Schevtchenkella* spp.

In the textual descriptions of the type species of genera *Spinacarus* (*S. guniujiangensis* Xue et al. 2009) and *Quadratum* (*Q. glaberi* Huang & Wang 2004), the dorsal opisthosoma is described as having a "shallow median furrow", but such topography is absent in the drawings: Figure 10D [50] and Figure 1A [49], respectively.

Genus *Calacarus* Keifer 1940

= *Phetehaburus* Amrine, Stasny & Flechtmann 2003: 70; synonymy by Qin et al. 2021: 262 [51].

Diagnosis. All leg and opisthosomal setae present except *l*'' II, tubercles of *sc* present or absent, opisthosomal annuli more or less distinctly dorsoventrally differentiated, opisthosoma with three or five longitudinal dorsal ridges usually bearing wax, most species are purple and produce wax forming bands on opisthosomal ridges and prodorsal shield.

Number of species. About 50, *Calacarus* is the largest genus in Calacarini.

New combination. We found that *Calacarus mussaendae* Keifer 1977 [52] does not have setae *l*' I (present in *Calacarus*) and corresponds to the diagnosis of *Procalacarus.* We exclude it from *Calacarus* and propose a new combination: *Procalacarus mussaendae* (Keifer 1977) **n. comb.**

Calacarus baviensis **n. sp.**—Figures 3–5.

FEMALE (*n* = 9). Body fusiform, slightly yellowish, 223 (180–228), 90 (77–97) wide at level of setae *c2*. **Prodorsal shield** subtriangular, 55 (50–59), 70 (63–78) wide, extending over chelicerae bases, forming a broad, rounded frontal lobe with a slight indentation in the anterior edge. Scapular setae *sc* absent, their tubercles present, very small, rounded, situated ahead of rear shield margin. Shield ornamentation (Figures 3A and 4B–D) consists of wax-bearing ridges (further called "lines" for simplicity) forming distinct cells in medial and lateral zones (MZ and LZ sensu Figure 2A). Median line is incomplete, present only on frontal lobe separating two distinct rounded cells. Admedian lines are complete, entire, and sinuate. MZ with two rounded apical cells on frontal lobe followed by area of about ¾ shield length, flanked by admedian lines, without median line and cells, anteriorly subtriangular, followed by narrow, vertical band up to broadly bilobed, subcordial area basally. LZ with a row of four rounded cells subequal in size on each side of prodorsal shield; the inner (the 1st) cell of LZ closed, not connected with admedian line, other cells partially and weakly separated from each other, with outer (the 4th) cell posteriorly closed. Submedial zone (SMZ sensu Figure 2A) smooth or with sparse indistinct microtubercles, without lines and cells, with *sc* tubercles near the lateral lobes of the subcordial area of MZ. Epicoxal area with microtubercles and dashes partly arranged in rows. **Gnathosoma** projecting downward, palps 32 (29–35). Gnathosomal setae: seta *ν* 3 (2–4); pedipalp genual seta *d* 10 (10–13);

pedipalp coxal seta *ep* 3 (2–4). Suboral plate with dashes and microtubercles, 11 (10–14), 30 (29–31) wide.

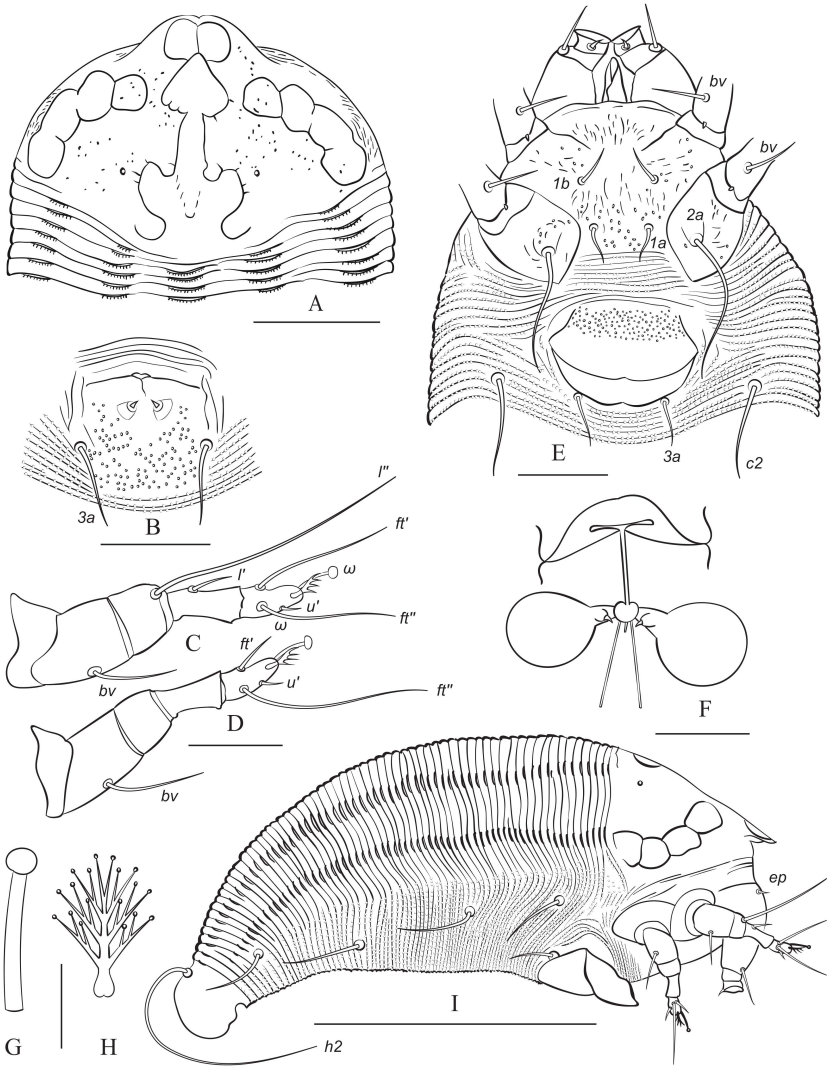

Figure 3. Drawings of *Calacarus baviensis* **n. sp.** (**A**)—prodorsal shield, (**B**)—male genital area, (**C**)—leg I, (**D**)—leg II, (**E**)—coxigenital area, (**F**)—female internal genitalia, (**G**)—tarsal solenidion I, (**H**)—empodium I, (**I**)—semilateral view of female. Scale bar: (**A**)—25 μm; (**B**,**E**)—15 μm, (**C**,**D**,**F**)—10 μm; (**G**,**H**)—5 μm; (**I**)—100 μm.

Leg I 34 (28–36), tarsus 6 (6–8), *u′* 4 (4–5), *ft′* 17 (15–20), *ft″* 22 (20–24), *ω* 5 (5–7) with large spherical knob; empodium 4 (4–5), 4-rayed, all rays except terminal pair with one (in subapical ray) or two (in other rays) subrays; tibia 5 (5–8), *l′* 4 (4–9); genu 5 (4–6), *l″* 29 (29–34), femur 12 (10–13), *bv* 13 (11–13). **Leg II** 30 (26–32), tarsus 7 (5–7), *u′* 3 (3–4), *ft′* 4 (4–7), *ft″* 19 (16–24), *ω* 7 (6–7) with large spherical knob; empodium 4 (4–5), 4-rayed, similar to empodium I; tibia 7 (5–7); genu 3 (3–5), *l″* absent, femur 13 (9–13), *bv* 10 (7–12). **Coxal plates I and II** with thin striae and small rounded microtubercles that are present on largely entire coxal plates I, and on coxal plates II mainly near tubercles of setae *2a*; coxal setae *1b* 9 (7–10), 15 (13–16) apart; *1a* 11 (7–12), 14 (10–14) apart; *2a* 25 (23–31), 33 (28–34) apart. Prosternal apodeme very indistinct; 13 (10–13) incomplete coxigenital annuli between coxae II and epigynium.

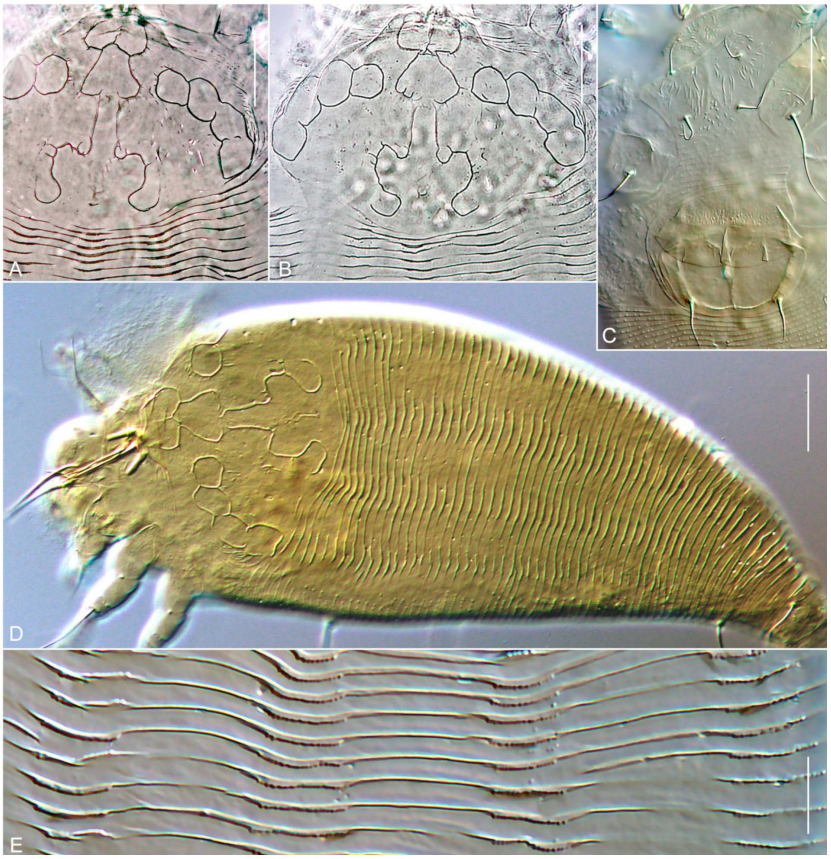

Figure 4. PC LM (**A**,**B**) and DIC LM (**C**–**E**) microphotographs of *Calacarus baviensis* **n. sp.** (**A**)—male prodorsal shield, (**B**)—female prodorsal shield, (**C**)—female coxigenital area, (**D**)—dorsolateral view of a female, (**E**)—rows of denticulate plates on longitudinal opisthosomal ridges. Scale bar: (**A**,**B**,**D**) = 20 μm; (**C**) = 15 μm; (**E**) = 3 μm.

External genitalia. Genital coverflap posteriorly rounded and slightly notched midway, smooth distally and microtuberculated basally, 14 (10–16), 27 (24–30) wide; setae *3a* 7 (6–7), 17 (16–19) apart. **Internal genitalia (*n* = 3).** Spermathecae subspherical, 10–14 wide; spermathecal duct short, about 2, 2–3 wide, with small acuminate spermathecal process; longitudinal bridge 13–16; anterior genital apodeme bell-shaped, oblique apodeme distinct; two narrow genital tubules about 12–15 long each join genital vestibulum posteriorly in all studied females, extending posteriad (Figure 5).

Opisthosoma dorsally with 63 (61–66) annuli, ventrally with 81 (73–84) annuli between posterior margin of coxae II and caudal lobes. Dorsal annuli form five distinct longitudinal ridges that are present from rear margin of prodorsal shield to the posteriormost caudal annulus but more pronounced in anterior 2/3 of body. These ridges are formed by narrow rounded plates with tiny marginal denticles (Figure 4E). Setal lengths: *c2* 23 (14–32), *d* 38 (27–46), *e* 23 (22–26), *f* 19 (19–21); *h1* absent; *h2* 32 (32–44); 16 (12–16) annuli from rear shield margin to *c2*; 19 (18–24) annuli between *c2*–*d*; 24 (21–25) annuli between *d* and *e*; 16 (14–17) annuli between *e* and *f*; 6 (5–8) annuli between *f* and *h2*.

MALE (*n* = 1). Body fusiform, slightly yellowish, 171, 71 wide at level of setae *c2*. **Prodorsal shield** subtriangular, 47, 64 wide. Prodorsal shield pattern similar to that in females. **Gnathosoma** projecting downward, palps 28. Gnathosomal setae: seta *v* 2; pedipalp genual seta *d* 10; pedipalp coxal seta *ep* 3. Suboral plate 11, 33 wide. **Leg I** 27, tarsus 6, *u′* 3, *ft′* 14, *ft″* 18, *ω* 5 with large spherical knob; empodium 5, 4-rayed; tibia 8, *l′* 4; genu5, *l″* 26, femur 10, *bv* 10. **Leg II** 27, tarsus 5, *u′* 3, *ft′* 4, *ft″* 16, *ω* 5 with large spherical knob; empodium 5, 4-rayed; tibia 7; genu 4, *l″* absent, femur 9, *bv* 9. **Coxal plates I and II** with thin striae and rounded microtubercles; coxal setae *1b* 11, 8 apart; *1a* 9, 10 apart; *2a* 25, 24 apart. Prosternal apodeme indistinct; 10 incomplete coxigenital annuli between coxae II

and epigynium. **Genital area** 18, 16 wide; setae *3a* 7, 17 apart, setae *eu* about 0.5, cuticle between tubercles of *3a* microtuberculated. **Opisthosoma** dorsally with 60 smooth annuli forming five longitudinal ridges with wax, ventrally with 63 microtuberculated annuli between posterior margin of coxae II and caudal lobes. Setal lengths: *c2* 17, *d* 35, *e* 19, *f* 17; *h1* absent; *h2* 30; 9 annuli from rear shield margin to *c2*; 15 annuli between *c2*–*d*; 18 annuli between *d* and *e*; 14 annuli between *e* and *f*; 7 annuli between *f* and *h2*.

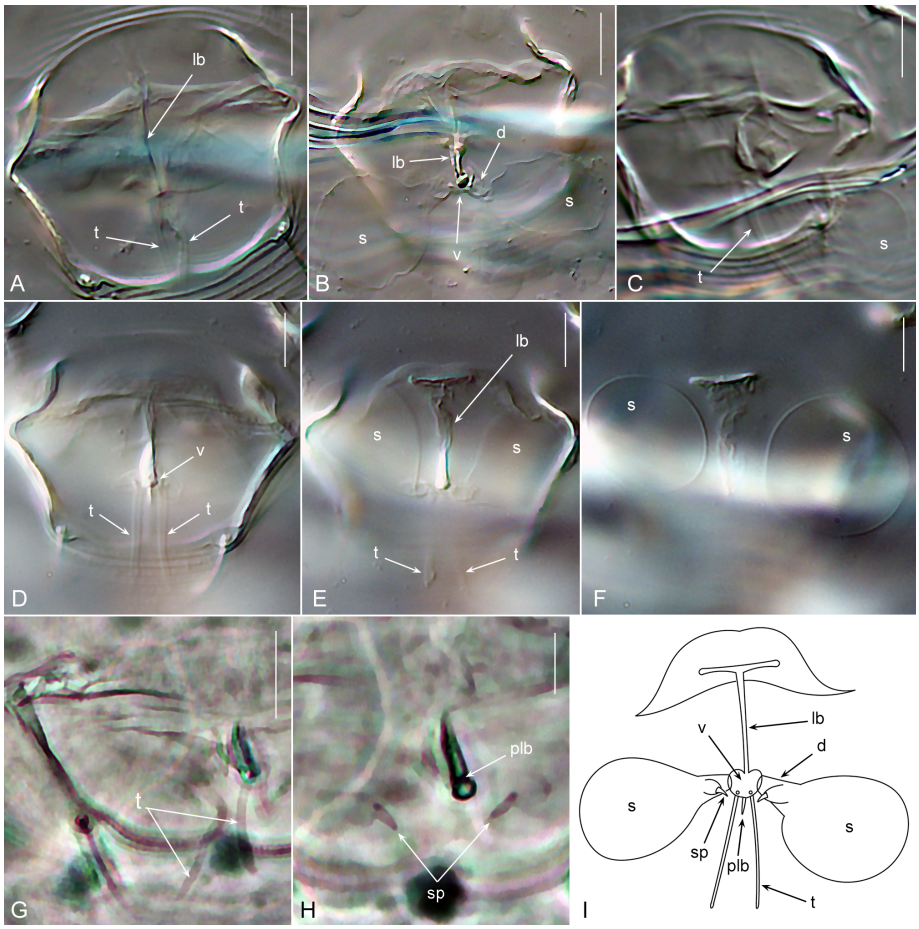

Figure 5. DIC LM (**A**–**F**) and PC LM (**G**,**H**) microphotographs of internal genitalia in four females (A+B; C; D+E+F; G+H) of *Calacarus baviensis* **n. sp.** and simplified scheme of spermathecal apparatus (**I**). Intact spermathecae (s) are drop-shaped, directed laterad, and slightly posteriad (**B**). In over-flattened slide-mounted females, spermathecae may be displaced anteriad (**E**,**F**). Short funnel-like spermathecal duct (d) connects spermatheca medially with vestibulum (v), that is a small fovea in posterior genital cuticle covered by distal part of genital coverflap and leading to the genital slit formed by plates of longitudinal bridge (lb) (**A**,**B**,**D**,**E**). Two small thorn-like spermathecal processes (sp) are situated in distal spermathecal duct; they resemble a pair of tiny setae (similar to male *eu*), directed convergently midway, and can be observed near posterior part of longitudinal bridge (plb) (**H**). Two narrow genital tubules (t) extending backward from vestibulum (v); both of them can be seen in most intact females (**A**,**D**,**E**,**G**), and in deformed females only one of them can be rarely detected (**C**). Scale bar: (**A**–**G**) = 5 μm, (**H**) = 2 μm.

Type material. Holotype female on slide E4724 and paratype females in slide series E4725–E4741 and in vials filled with 96% ethanol collected on 17 February 2023 by P.E. Chetverikov, N. Le, and V.D. Nguyen from upper leaf surface of *Mallotus paniculatus* (Lam.) Müll. Arg. (Euphorbiaceae), in Ba Vi National Park, 21°05′03.4″ N 105°23′00.9″ E (Vietnam, Figure 1). Type material is deposited in the Acarological Collection of the Zoological Institute of the Russian Academy of Science (ZIN RAS) in Saint-Petersburg (Russia).

Host and relation to host. Mites live on the upper surface of leaves of *Mallotus paniculatus* (Lam.) Müll. Arg. (Euphorbiaceae), a woody plant with large subrhomboidal leaves that is widely distributed in India, China, South-Eastern Asia and Australia (Figure 1B,D), causing no visible damage.

Etymology. The species name, *baviensis*, is an adjective of the masculine gender. It is derived from "Ba Vi", the name of the national park in northern Vietnam where this species was found for the first time.

Differential diagnosis. The new species is morphologically closest to *C. burchelliae* n. sp., *C. caricae* (Qin et al. 2019 [53]), and *C. liquidambarus* (Qin et al. 2021 [51]). The main differences between them are in (a) ornamentation of prodorsal shield and genital coverflap, (b) number of empodial rays, and (c) shape of the plates forming longitudinal opisthosomal ridges (Table 3).

Table 3. Morphological differences between five *Calacarus* species.

Character	*C. baviensis* n. sp.	*C. burchelliae* n. sp.	*C. caricae* (Qin et al. 2019)	*C. liquidambarus* (Qin et al. 2021)	*C. speciosissimum* Flechtmann 1999
Median line of prodorsal shield	absent (except separation between two anteriormost cells of MZ)	weak, present in posterior half of prodorsal shield	absent	very short, present only near posterior margin of prodorsal shield	weak, present in posterior half of prodorsal shield
Connection between admedian line and first (inner) lateral cell	absent	present	present	present	present
Connections between admedian lines posterior to frontal lobe	absent	short transverse line in the center and V-shaped line in posterior half of shield	short transverse line in the center and rounded V-shaped line in posterior half of shield	V-shaped line in posterior half of shield	Y-shaped line in the center and rounded V-shaped line in posterior half of shield
Number of empodial rays	4	6	6	5	6
Shape of plates forming longitudinal opisthosomal ridges	entire, falcate, with marginal denticles	subdivided into two small plates, without denticles	entire, falcate, without denticles	entire, falcate, without denticles	entire, falcate, with marginal denticles
Ornamentation of genital coverflap	smooth distally and microtuberculated basally	with ~30 short thin striae distally and microtuberculated basally	smooth distally and microtuberculated basally	"with granules at base and a distal row of short lines"	with short faint longitudinal lines in two ranks
Type locality (country)	Vietnam	South Africa	Laos	South China	French Antilles, Brazil
Host plant	*Mallotus paniculatus* (Lam.) Müll. Arg. (Euphorbiaceae)	*Burchellia bubalina* (L.f.) Sims (Rubiaceae)	*Carica papaya* L. (Caricaceae)	*Liquidambar formosana* Hance (Altingiaceae)	*Clerodendron speciosissimum* Drapiez *(Verbenaceae), Capsium* spp. *(Solanaceae)*
Relation to host	vagrant on upper leaf surface causing no visible damage	vagrant on lower leaf surface causing no visible damage	vagrant on lower leaf surface causing yellowing, curling and shrinking of leaves	vagrant on lower leaf surface causing no visible damage	vagrant on leaves causing no visible damage
Reference	This paper	This paper	[51,53]	[53]	[46]

Calacarus burchelliae **n. sp.**—Figures 6–8.

FEMALE (*n* = 8). Body fusiform, slightly yellowish, 223 (206–244), 99 (82–99) wide at level of setae *c2*. **Prodorsal shield** subtriangular, 60 (51–60), 80 (71–88) wide, extending over chelicerae bases, forming a broad, rounded frontal lobe with a slight indentation in the anterior edge. Scapular setae *sc* absent, their tubercles present, very small, rounded, situated ahead of rear shield margin. Shield ornamentation (Figures 6B and 7A–E) consists of wax-bearing ridges (further called "lines" for simplicity) forming distinct cell-like pattern in medial and lateral zones (MZ and LZ sensu Figure 2A). Anterior half of MZ with two distinct rounded cells on frontal lobe, with tiny microtubercles, followed by large anteriorly broad and posteriorly narrow subrectangular cell; posterior half of MZ with large elongated

subrhomboidal cell subdivided by weak median line into two narrow mirrored cells, and basal distinct M-shaped figure containing distinct inner V-shape closing the large subrhomboidal cell posteriorly. LZ with a row of five cells on each side of prodorsal shield; the inner cell anteriorly open, the next one subpentagonal and closed, all other cells subrectangular, partially separated from each other. Submedial zone (SMZ sensu Figure 2A) smooth, with *sc* tubercles about 5–7 μm ahead of rear shield margin. Very weak traces of 3–4 large cells occupying entire SMZ in some specimens, hardly discernible. Epicoxal area with microtubercles and dashes partly arranged in rows. **Gnathosoma** projecting downward, palps 32 (29–35), oral stylet angled, short-form. Gnathosomal setae: seta *ν* 2 (2–3); pedipalp genual seta *d* 9 (9–10); pedipalp coxal seta *ep* 4 (3–5). Suboral plate with microtubercles, 12 (10–12), 26 (23–26) wide.

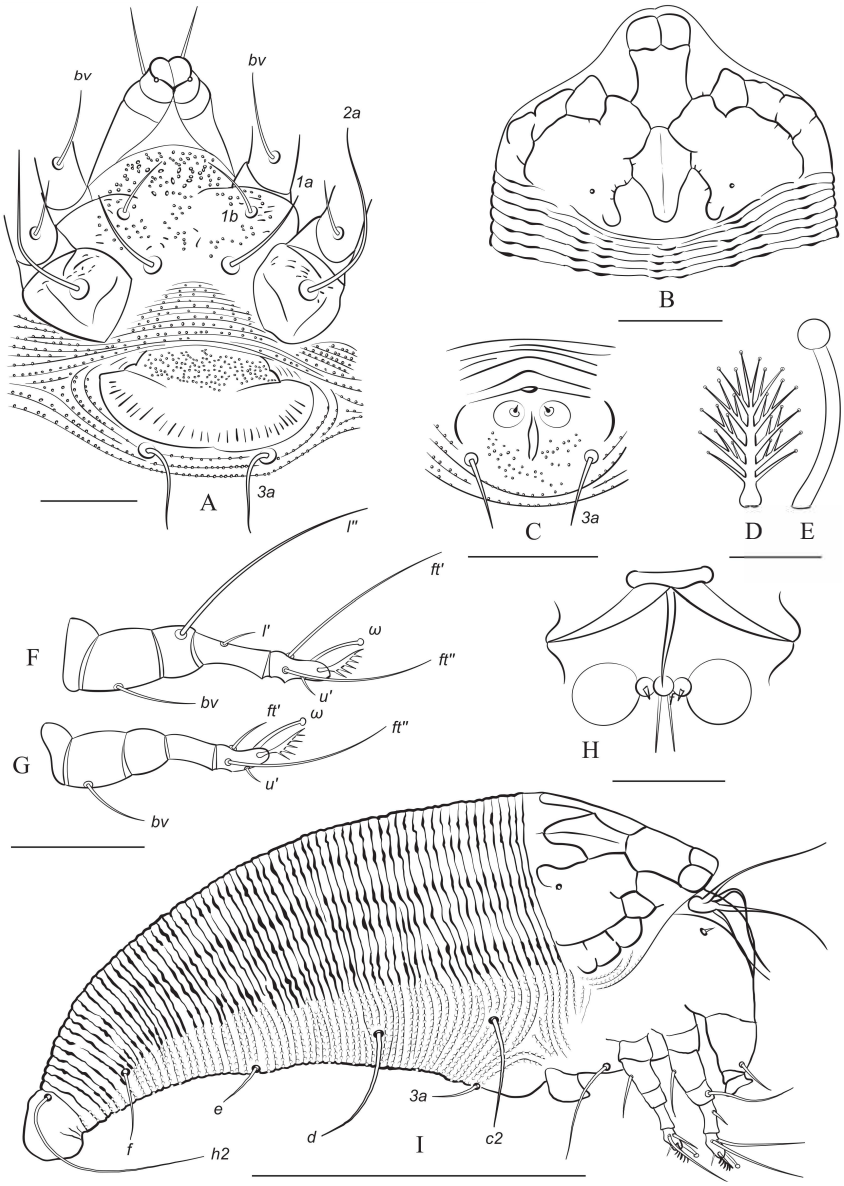

Figure 6. Drawings of *Calacarus burchelliae* **n. sp.** (**A**)—coxigenital area, (**B**)—prodorsal shield, (**C**)—male genital area, (**D**)—empodium I, (**E**)—tarsal solenidion I, (**F**)—leg I, (**G**)—leg II, (**H**)—female internal genitalia, (**I**)—semilateral view of female. Scale bar: (**A**,**C**)—15 μm; (**B**,**F**,**G**)—20 μm; (**D**,**E**)—4 μm; (**H**)—10 μm; (**I**)—115 μm.

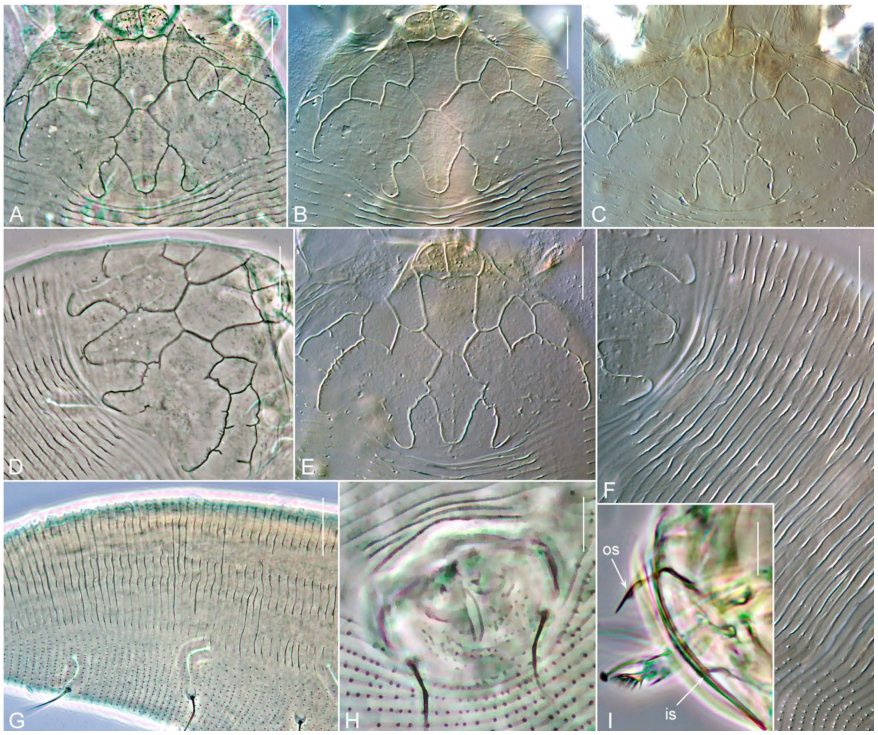

Figure 7. PC LM (**A**,**D**,**G**–**I**) and DIC LM (**B**,**C**,**E**,**F**) microphotographs of *Calacarus burchelliae* **n. sp.** (**A**–**E**)—variation of prodorsal shield ornamentation in females; (**F**,**G**)—longitudinal opisthosomal ridges, (**H**)—male external genitalia, (**I**)—oral (os) and infracapitular (is) stylets. Scale bar: (**A**–**G**) = 15 μm, (**H**) = 5 μm, (**I**) = 10 μm.

Leg I 36 (34–36), tarsus 8 (7–10), *u′* 4 (4–5), *ft′* 22 (19–24), *ft″* 23 (23–25), *ω* 8 (7–10) with large spherical knob; empodium 5 (5–7), 6-rayed, all rays except terminal and basal pairs with two subrays; tibia 9 (8–10), *l′* 6 (4–6); genu 6 (4–6), *l″* 32 (32–35), femur 12 (11–13), *bv* 14 (10–15). **Leg II** 34 (31–34), tarsus 7 (7–8), *u′* 5 (3–6), *ft′* 6 (5–9), *ft″* 21 (20–24), *ω* 8 (8–9) with large spherical knob; empodium 5 (5–7), 6-rayed, similar to empodium I; tibia 8 (6–9); genu 5 (4–6), *l″* absent, femur 12 (10–12), *bv* 12 (9–14). **Coxal plates I and II** with distinct spine-shaped microtubercles situated mainly around tubercles of setae *1a* and *2a*; coxal setae *1b* 8 (7–9), 16 (15–18) apart; *1a* 14 (12–16), 11 (10–12) apart; *2a* 39 (36–47), 30 (30–34) apart. Prosternal apodeme indistinct; 13 (10–13) incomplete coxigenital annuli between coxae II and epigynium.

External genitalia. Genital coverflap posteriorly rounded and slightly notched midway, a row of short subparallel dashes along distal margin of genital coverflap, pregenital area covered with microtubercles and very short dashes, 13 (12–15), 29 (27–31) wide; setae *3a* 13 (12–15), 17 (17–21) apart. **Internal genitalia (*n* = 3).** Spermathecae spherical, 7–9 wide; spermathecal tubes very short, subspherical, about 2–3 in diameter, with small acuminate spermathecal process about 0.5–1; genital tubule 5–8 long, longitudinal bridge 9–12; anterior genital apodeme trapezoidal, bell-shaped, smooth, oblique apodeme distinct, straight.

Opisthosoma dorsally with 69 (60–70) annuli, ventrally with 80 (71–80) annuli between posterior margin of coxae II and caudal lobes. Dorsal annuli smooth except posterior-most 5–10 annuli with small irregular round microtubercles. Ventral annuli with spine-shaped microtubercles in anterior half of opisthosoma and more elongated, gradually becoming ridge-like posterior to tubercles of setae *f*. Setal lengths: *c2* 25 (20–25), *d* 52 (47–62), *e* 20 (18–20), *f* 21 (20–24); *h1* absent; *h2* 47 (46–63); 14 (12–16) annuli from rear shield margin to *c2*; 18 (15–18) annuli between *c2*–*d*; 20 (18–20) annuli between *d* and *e*; 20 (16–20) annuli between *e* and *f*; 8 (7–8) annuli between *f* and *h2*.

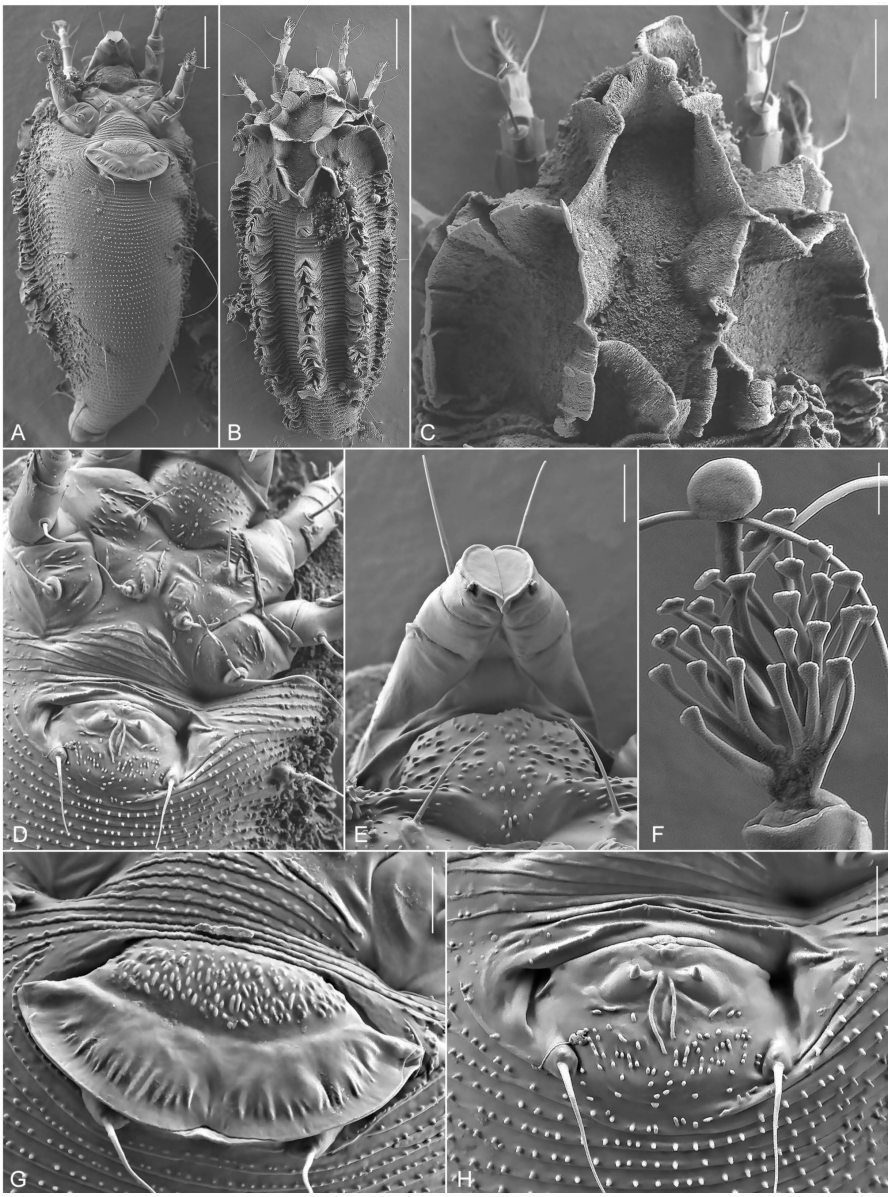

Figure 8. LT-SEM images of *Calacarus burchelliae* **n. sp.** (**A**,**B**)—dorsal (**A**) and ventral (**B**) view of entire female; (**C**)—prodorsal shield; (**D**)—male coxigenital area; (**E**)—ventral view of gnathosoma, (**F**)—empodium I and tarsal solenidion I; (**G**)—female external genitalia; (**H**)—male external genitalia. Photographs—C. Craemer. Scale bar: (**A**,**B**) = 20 μm; (**C**) = 10 μm; (**D**,**E**,**H**) = 4 μm; (**F**) = 1 μm; (**G**) = 5 μm.

MALE (*n* = 1). Body fusiform, slightly yellowish, 174, 63 wide at level of setae *c2*. **Prodorsal shield** subtriangular, 47, 73 wide. Prodorsal shield pattern similar to that of female. **Gnathosoma** projecting downward, palps 40. Gnathosomal setae: seta *v* 2; pedipalp genual seta *d 9*; pedipalp coxal seta *ep 4*. Suboral plate 9, 21 wide. **Leg I 36**, tarsus 7, *u′* 3, *ft′* 20, *ft″* 22, *ω* 5 with large spherical knob; empodium 6, 6-rayed; tibia 8, *l′* 5; genu 5, *l″* 27, femur 12, *bv* 10. **Leg II 30**, tarsus 7, u′ 3, *ft′* 5, *ft″* 20, *ω* 7 with large spherical knob; empodium 5, 6-rayed, similar to empodium I; tibia 9; genu 4, *l″* absent, femur 11, *bv* 8. **Coxal plates I and II** microtuberculated, coxal setae *1b* 14, 9 apart; *1a* 9, 13 apart; *2a* 26, 35 apart. Prosternal apodeme indistinct; 10 incomplete coxigenital annuli between coxae II and epigynium. **Genital area** 15, 12 wide, setae *3a* 13, 14 apart, *eu* 0.5, microtubercles on area between setae *3a*, two more or less parallel ridges starting between *eu* setal tubercles up to level of setae *3a*, two rounded transversal ridges on the basal margin of large, rounded relatively flat setal tubercles of *eu* (observed only under LT-SEM).

Opisthosoma dorsally with 63 annuli, ventrally with 73 annuli between posterior margin of coxae II and caudal lobes. Dorsal annuli smooth except posterior-most 5–10 annuli with small irregular round microtubercles. Ventral annuli microtuberculated similar to those in females. Setal lengths: *c2* 20, *d* 46, *e* 19, *f* 19; *h1* absent; *h2* 38; 12 annuli from rear shield margin to *c2*; 15 annuli between *c2*–*d*; 16 annuli between *d* and *e*; 17 annuli between *e* and *f*; 8 annuli between *f* and *h2*.

Type material. Holotype female on slide E4753, paratypes in slide series E4754–E4766 collected on 12 November 2016 by P. Chetverikov, C. Craemer, and S. Neser from lower leaf surface of *Burchellia bubalina* (L.f.) Sims (Rubiaceae) near Kirstenbosch National Botanical Gardens, 33°59′09.4″ S 18°26′01.7″ E, Cape Town, South Africa (Figure 1D). Type material is deposited in the Acarological Collection of the Zoological Institute of the Russian Academy of Science (ZIN RAS) in Saint-Petersburg (Russia) and ARC Plant Protection Research Institute at Roodeplaat, Pretoria (South Africa).

Host and relation to host. Mites live on the lower surface of leaves of *Burchellia bubalina* (L.f.) Sims (Rubiaceae) causing no visible damage.

Additional material. Females in slide series E4746–E4748 collected on 6 November 2016, same collectors, host plant and relation to host, mountain road from Avontuur to Knysna near bridge over the Dieprivier, 33°51′38.3″ S 23°10′23.0″ E, South Africa (Figure 1D).

Etymology. The species name, *burchelliae*, is a noun, gender feminine, in genitive case. It is derived from the generic name of the host plant, *Burchellia*.

Differential diagnosis. *Calacarus burchelliae* **n. sp.** is close to *C. baviensis* **n. sp.** and *C. speciosissimum* Flechtmann 1999. Contrary to these two species, *C. burchelliae* **n. sp.** has a large subrhomboidal cell containing a weak median line in the posterior part of the MZ (two elongate cells in *C. speciosissimum* and opened subcordial figure in *C. baviensis* **n. sp.**). Other differences are listed in Table 3.

***Calacarus pusillus* Pye 2012**

Calacarus pusillus Pye 2012: 50–52. Figures 4–6.

Remarks. This species was described from Great Britain from *Calluna vulgaris* (L.) Hill (Ericaceae) as vagrant on leaves, causing no visible damage to host [12]. We found this species in Latvia and northwest Russia (Table 1) on the same host (no visible damage) accompanied by *Aceria exigua* (Liro 1940). Our findings of *C. pusillus* represent the most northern localities for this species and for the entire tribe Calacarini. Considering the wide distribution of the host plant, *Calluna vulgaris*, in Eurasia [35] and its introduction to Canada, the USA, New Zealand, and Tasmania [31], future field surveys may reveal the distribution of *Calacarus pusillus* being as broad as that of its host.

***Jiangsuacarus* sp.**

Remarks. Among ethanol materials kept in our collections, we have vials containing purple-colored mites from a grass *Arundinaria gigantea* collected in May 2019 in the USA (see Table 1 for detail). Examination of slide-mounted specimens showed that these mites lack setae *sc* and their tubercles, have all common leg and opisthosomal setae except *h1*, and fit the diagnosis of the genus *Jiangsuacarus* Xue 2009 which, in turn, is very close to genus *Jaranasia* Chandrapatya & Boczek 2000. According to the original description [54], the type species *Jaranasia anamensiae* Chandrapatya & Boczek 2000, retains leg setae *l*″ II. Following this concept of *Jaranasia*, the genus *Jiangsuacarus* should have been synonymized with *Jaranasia*. However, Chandrapatya et al. [47] recently published new drawings of *J. anamensiae* showing an absence of *l*″ II. We checked the two paratypes of this species deposited in the collection of J.W. Amrine and confirm absence of *l*″ II. Therefore, *Jaranasia* and *Jiangsuacarus* are two valid genera separated by the presence (in *Jiangsuacarus*)/absence (in *Jaranasia*) of setae *l*″ II.

New combination. Besides the type species, genus *Jaranasia* includes only one more species, *J. sesleriae* Skoracka 2004, which was found as vagrant on upper leaf surfaces of two grasses (*Sesleria varia* (Jacq.) Wettst. and *Festuca rubra* L.) in Poland [55]. Drawings of this species from its original description unambiguously show the presence of *l*″ II. Therefore, we propose a new combination: *Jiangsuacarus sesleriae* (Skoracka 2004) **n. comb.** (transferred from *Jaranasia*).

***Genus Paracalacarus* Keifer 1962**

***Paracalacarus podocarpi* Keifer 1962**

Paracalacarus podocarpi Keifer, 1962: 7–8; Ehara, 1993: 138–139; Kuang, 1995: 79–80; Hong & Zhang, 1996: 38–39; Song et al. 2008: 16.

Remarks. Up to now, this species has been reported from the USA, China, and Japan from *Podocarpus* spp. (Podocarpaceae) [56–59]. Since *P. podocarpi* is absent in the recently published comprehensive catalog of eriophyoid mites of Brazil [60], our finding of *P. podocarpi* on an introduced ornamental *Podocarpus* sp. (Table 1) is the first record of this species in Brazil and in South America. Morphologically, *P. podocarpi* is very similar to *Paraphetehaburus cephalotaxus* Xue et al. 2007 described from *Cephalotaxus fortunei* (Cephalotaxaceae); however, in *P. podocarpi*, the tubercles of *sc* are present contrary to *P. cephalotaxus* (the tubercles of *sc* are absent) [61]. Besides this difference, *P. cephalotaxus* and *P. podocarpi* are very similar. Synonymy of *Paraphetehaburus* and *Paracalacarus* needs testing based on molecular phylogenetic methods.

Similar to our previous observations on *Phyllocoptes bilobospinosus* and *Aberoptus schotiae* [14,62], in slide-mounted females of *P. podocarpi* that were incompletely cleared, we observed outlines of anal glands filled with vesicles (Figure 2F). This is the first documented report of the elements of the recently described anal secretory apparatus (ASA sensu [14]) in Calacarini.

The vial with mites *P. podocarpi* in 96% ethanol was stored in a freezer at −20 °C in 2015–2019; however, later (2019–2023), it was by mistake kept at room temperature (+25 °C). Probably due to this, we failed to obtain positive results for the *Cox1* gene; however, from one of four DNA extractions, we obtained a high-quality partial sequence of the *28S* gene which showed ~99% identity with the conspecific sequence KM111070 [63] from China. Therefore, in some cases, fragments of *rDNA* genes can be successfully amplified even based on the DNA extracted from eriophyoid mites that were stored about four years at room temperature. This is in accordance with the previously reported experience of successful amplification of short fragments of *18S rDNA* gene from DNA that was extracted from mummies of eriophyoid mites from old (10–15 years) dry plant material kept in envelopes at room temperature for years (J.A., unpublished observation).

***Genus Viginticus* Duarte & Navia 2020**

***Viginticus searsiae* n. sp**.—Figures 9 and 10.

FEMALE (*n* = 9). Body fusiform, slightly yellowish, 273 (225–277), 101 (92–108) wide at level of setae *c2*. **Prodorsal shield** subrhomboidal, 68 (63–70), 90 (76–90) wide, extending over cheliceral bases, forming a broad, rounded frontal lobe. Scapular setae *sc* absent, *sc* tubercles present, relatively small, rounded, ahead of rear shield margin. Shield ornamentation (Figure 9B,I and Figure 10A,C) consists of wax-bearing ridges (further called "lines" for simplicity) forming distinct cell-like pattern in medial, lateral, and submedial zones (MZ, LZ, SMZ sensu Figure 2A). Median line complete, present from anterior margin of the frontal lobe to rear shield margin, faint, except strong keel-like apical part separating two rounded cells on frontal lobe. Admedian lines complete, entire, sinuate. MZ with eight cells in two vertical rows; two basally opened posterior cells and two closed apical cells on frontal lobe are relatively short; four medial cells of MZ elongate. LZ with five subrectangular cells on each side of prodorsal shield. SMZ with five closed large cells, the lateralmost of them elongated, oblique, subparallel and adjacent to the row of cells of LZ; two weak subparallel short lines going from *sc* tubercles to rear shield margin. All cells

in the LZ with relatively densely distributed short dashes and microtubercles; and the remainder of the shield with more sparsely distributed microtubercles. Epicoxal area with microtubercles and dashes partly arranged in rows. **Gnathosoma** projecting downward, palps 41 (40–42), palpcoxal base with microtubercles latero-ventrally. Gnathosomal setae: seta *ν* 3 (2–3); pedipalp genual seta *d* 10 (10–12); pedipalp coxal seta *ep* 4 (3–4). Suboral plate with numerous small microtubercles, 16 (16–17), 28 (27–28) wide.

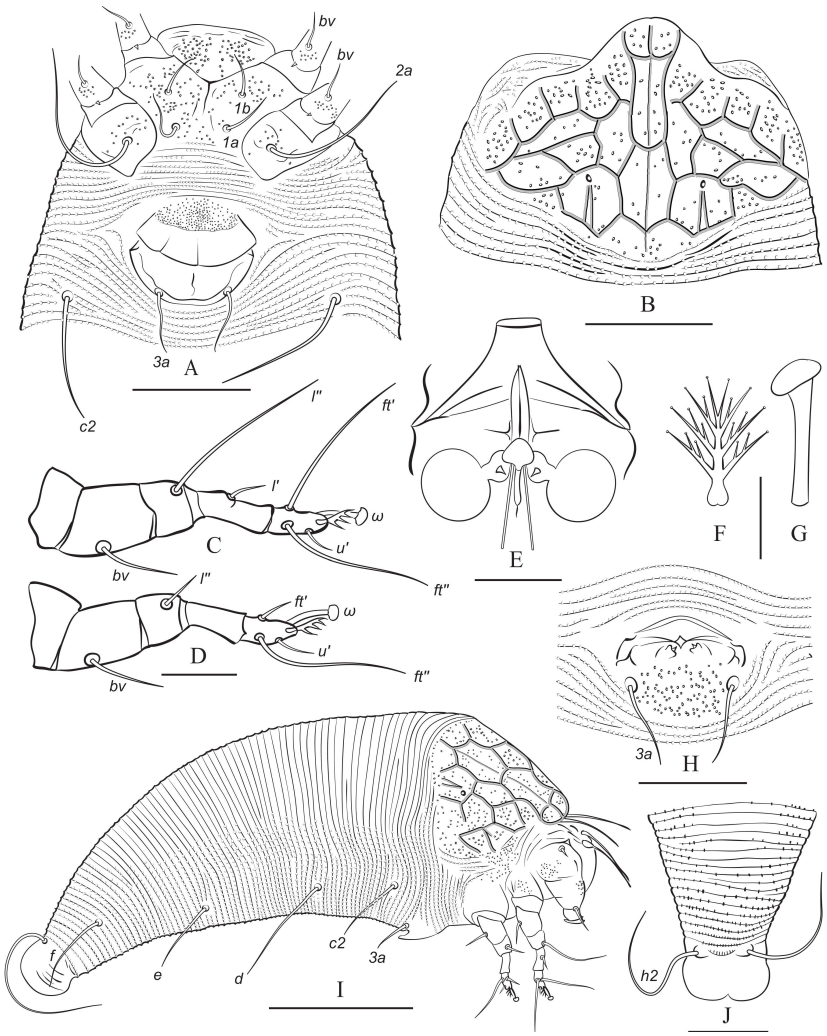

Figure 9. Drawings of *Viginticus searsiae* **n. sp.** (**A**)—coxigenital area, (**B**)—prodorsal shield, (**C**)—leg I, (**D**)—leg II, (**E**)—female internal genitalia, (**F**)—empodium I, (**G**)—tarsal solenidion I, (**H**)—male genital area, (**I**)—semilateral view of female, (**J**)—dorsal view of telosome. Scale bar: (**A**)—30 μm; (**B**)—40 μm; (**C**–**E**)—10 μm; (**F**,**G**)—3 μm; (**H**,**J**)—20 μm; (**I**)—70 μm.

Leg I 42 (41–43), tarsus 8 (7–8), *u′* 5 (4–6), *ft′* 21 (19–22), *ft″* 23 (21–24), *ω* 7 (6–8) with large spherical knob; empodium 5 (4–6), 4-rayed, all rays except terminal pair with one (in sub-apical ray) or two (in other rays) subrays; tibia 10 (9–10), *l′* 4 (4–6); genu 6 (6–7), *l″* 29 (25–30), femur 13 (12–16), *bv* 13 (12–15). **Leg II** 39 (37–40), tarsus 8 (8–9), *u′* 3 (3–4), *ft′* 6 (5–6), *ft″* 21 (19–21), *ω* 8 (6–8) with large spherical knob; empodium 5 (5–6), 4-rayed, similar to empodium I; tibia 8 (7–9); genu 6 (6–7), *l″* 21 (19–21), femur 12 (12–14), *bv* 11 (10–12). **Coxal plates I and II** with microtubercles; coxal setae *1b* 21 (20–21), 11 (10–15) apart; *1a* 15 (14–15), 15 (14–18) apart; *2a* 41 (33–43), 40 (31–40) apart. Prosternal apodeme about 5 (4–7); 13 (10–13) incomplete coxigenital annuli between coxae II and epiginium.

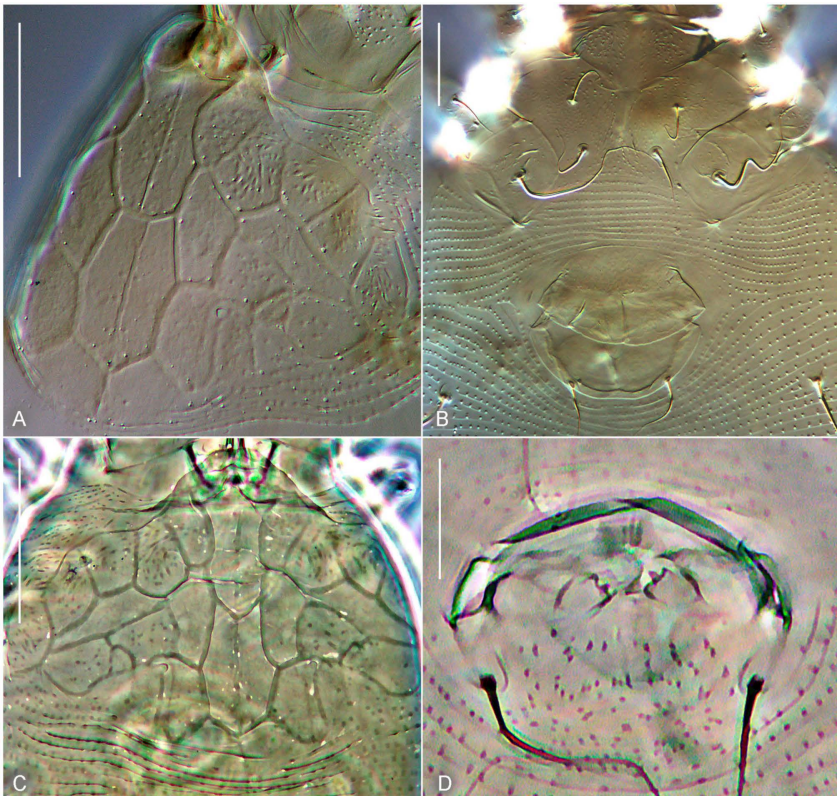

Figure 10. DIC LM (**A**,**B**) and PC LM (**C**,**D**) microphotographs of *Viginticus searsiae* **n. sp.** (**A**,**C**)—female (**A**) and male (**C**) prodorsal shields, (**B**)—female coxigenital area, (**D**)—male external genitalia. Scale bar: (**A**–**C**)—30 μm; (**D**)—10 μm.

External genitalia. Genital coverflap posteriorly rounded and slightly notched midway, distal thin part of coverflap smooth, thicker basal part microtuberculated, 15 (12–12), 33 (30–35) wide; setae *3a* 19 (16–19), 19 (15–19) apart. **Internal genitalia (*n* = 3,** Figure 9E). Spermathecae subspherical, 7–10 in diameter wide; spermathecal tubes short, 2–3 wide, slightly swollen near vestibulum, with very small acuminate spermathecal process about 0.5; longitudinal bridge 9–12, with carina-like part protruding posterior to vestibulum; anterior genital apodeme trapezoidal, bell-shaped, smooth, oblique apodeme distinct, straight; two narrow, genital tubules join vestibulum posteriorly, extending posteriad, 10–12, about 0.5 wide.

Opisthosoma dorsally with 67 (63–71) annuli, ventrally with 89 (85–92) annuli between posterior margin of coxae II and caudal lobes. Dorsal annuli smooth except posterior-most 5–10 annuli with small irregular round microtubercles. Ventral annuli with spine-shaped microtubercles in anterior half of opisthosoma and more elongate, gradually changing in shape and becoming more elongate posteriad and eventually ridge-like posterior to tubercles of setae *f*. Setal lengths: *c2* 32 (25–34), *d* 57 (55–67), *e* 37 (37–47), *f* 30 (26–31); *h1* absent; *h2* 68 (47–68); 20 (19–21) annuli from rear shield margin to *c2*; 18 (16–19) annuli between *c2*–*d*; 24 (22–27) annuli between *d* and *e*; 20 (18–21) annuli between *e* and *f*; 7 (7–7) annuli between *f* and *h2*.

MALE (*n* = 3). Body fusiform, slightly yellowish, 227–238, 73–78 wide at level of setae *c2*. **Prodorsal shield** subtriangular, 59–65, 68–76 wide, anterior margin of prodorsal shield distinct, broadly rounded. Prodorsal shield pattern, shape, size and position of tubercles of *sc* similar to that in females. **Gnathosoma** projecting downward, palps 33–37. Gnathosomal setae: seta *ν* 2–3; pedipalp genual seta *d* 10–11; pedipalp coxal seta *ep* 3–4. Suboral plate 16–17, 21–25 wide.

Leg I 38-40, tarsus 7–8, *u′* 3–4, *ft′* 18–19, *ft″* 20–23, *ω* 6–7 with large spherical knob; empodium 4–4, 4-rayed, all rays except terminal pair with one (in subapical ray) or two

(in other rays) subrays; tibia 9–11, *l′* 3–4; genu 6-7, *l″* 23–27, femur 11–13, *bv* 11–13, with microgranulations ventro-distally. **Leg II** 33–38, tarsus 7–8, *u′* 3–4, *ft′* 4–5, *ft″* 19–21, *ω* 6–7 with large spherical knob; empodium 5–6, 4-rayed, similar to empodium I; tibia 8–8; genu 6–7, *l″* 5–9, femur 10–12, *bv* 10–12. **Coxal plates I and II** with distinct spine-shaped microtubercles situated mainly around tubercles of setae *1a* and *2a*; coxal setae *1b* 17–18, 10–12 apart; *1a* 12–13, 13–15 apart; *2a* 31–33, 34–40 apart. Prosternal apodeme indistinct; 10 incomplete coxigenital annuli between coxae II and epigynium. **Genital area** 18–20, 19–21 wide, setae *3a* 15–18, 13–16 apart, *eu* 0.5, microtubercles on area between setae *3a*.

Opisthosoma dorsally with 63–72 annuli, ventrally with 71–77 annuli between posterior margin of coxae II and caudal lobes. Dorsal annuli smooth except posterior-most 5–10 annuli with small irregular round microtubercles. Ventral annuli micotuberculated similar to those in females. Setal lengths: *c2* 24–24, *d* 51–60, *e* 33–43, *f* 21–27; *h1* absent; *h2* 49–52; 14–17 annuli from rear shield margin to *c2*; 14–15 annuli between *c2*–*d*; 19–20 annuli between *d* and *e*; 16–20 annuli between *e* and *f*; 7–8 annuli between *f* and *h2*.

Type material. Holotype female on slide E4776 and paratype females in slide series E4768–E4784 collected on 12 November 2016 by P. Chetverikov, C. Craemer, and S. Neser along veins on upper leaf surface of young leaves of *Searsia chirindensis* (Baker f.) Moffett (Anacardiaceae) near Kirstenbosch National Botanical Gardens (33°59′19.9″ S 18°25′56.1″ E), Cape Town, South Africa (Figure 1D) Type material is deposited in the Acarological Collection of the Zoological Institute of the Russian Academy of Science (ZIN RAS) in Saint Petersburg (Russia) and ARC Plant Protection Research Institute at Roodeplaat, Pretoria (South Africa).

Host and relation to host. Mites live on the upper surface of leaves of *Searsia chirindensis* (Baker f.) Moffett (Anacardiaceae) causing no visible damage.

Etymology. The species name, *searsiae*, is a noun, gender feminine, in genitive case. It is derived from the generic name of the host plant, *Searsia*.

Differential diagnosis. The new species is the second member of the recently established genus *Viginticus*. The main differences between *V. searsiae* **n. sp.** and *V. lupusmalum* Duarte & Navia 2020 (the type species of *Viginticus*) are in (a) number of opisthosomal annuli, (b) ornamentation of prodorsal shield and genital coverflap, (c) presence/absence of microtubercles on dorsal opisthosomal annuli, and (d) number of empodial rays (Table 4).

Table 4. Morphological differences between *V. searsiae* **n. sp.** and *V. lupusmalum* Duarte & Navia 2020.

Character	*V. searsiae* n. sp.	*V. lupusmalum*
Number of dorsal annuli	67 (63–71)	87 (77–89)
Number of ventral annuli	89 (85–92)	75 (67–79)
Number of empodial rays	4	5
Microtubercles on dorsal opisthosomal annuli	Absent	Present
Ornamentation of posterolateral zone of prodorsal shield	PLZ entirely occupied by five closed cells	PLZ with only two elongate cells adjacent to admedian line putatively formed by submedian I line
Shape and position of *sc* tubercles	Very small, microtubercle-like, situated within posteriormost closed cell of PLZ anterior to two subparallel short lines	Well-developed, situated outside of posterior part of submedian I line
Ornamentation of genital coverflap	Basally with microgranulations, distally smooth	Smooth
Type locality (country)	South Africa	Brazil
Host plant	*Searsia chirindensis* (Baker f.) Moffett (Anacardiaceae)	*Solanum lycocarpum* L. (Solanaceae)
Relation to host	Vagrant on upper leaf surface	Vagrant on leaves
Reference	This paper	[46]

3.2. Molecular Phylogenetics: Blast Searches and Cox1 and 28S Analyses

3.2.1. Blast Searches of Cox1 and D1D2 28S Sequences of Calacarini

Blast Searches of Calacarine Sequences from GenBank

Blastn searches of *Cox1* sequences revealed twelve 100% identical sequences assigned to five species of two calacarine and one diptilomiopine genera: *Latitudo* (MZ274906: *L. asiaticus* KK21b), *Calacarus* (MZ274905: *Calacarus* sp. FJ17), and *Diptilomiopus* (*D. stephanus*: MK516836; *D. keningaus*: MZ482693—MZ482700; *D. callicarpus*: MK516841).

Blastx searches of translated *Cox1* sequences showed that sequences of different species of *Jutarus* are close to those of mites from different eriophyid tribes (but not Calacarini), and sequence KM111104 of *Quadratum* sp. is notably closer to some *Epitrimerus*, *Diptacus*, and *Phyllocoptruta* (82–83% identity) than to any calacarine sequences (70–78% identity).

Blast searches of *D1D2 28S* sequences showed that sequence MZ288805 (*Calacarus carinatus* AH35) belongs to *Acaphylla*, sequences MZ288891 (*Calacarus* sp. GX30) and MZ288809 (*Calacarus* sp. FJ17) belong to Nothopodinae, MZ288814 (*Jaranasia* cf. *anamensiae* CQ3_1) belongs to a monocot-associated phyllocoptine, MZ288927 (*Neoshevtchenkella liquidambaris* GZ36) belongs to *Calacarus* (most probably *C. carinatus*), and MZ288889.1 (*Jutarus benjaminae* GX28) is 100% identical to four sequences of *Cecidophyes thailandica* (MZ288873, MZ288864, MZ288848, MZ288825).

Blast Searches of the New Sequences of Calacarines Obtained in This Study

Blastn/Blastx searches of *Cox1* sequences of calacarines from our material revealed the following most similar sequences of Eriophyoidea from GenBank (sorted by % identity): for OR756238 of *Calacarus baviensis* n. sp.—MZ483708 of *Tegonotus* sp. (56% coverage, 78.78% identity)/ULB32669 of *Rhyncaphytoptus* sp. (99% coverage, 87.99% identity); for OR756236 of *C. burchelliae* n. sp.—MW471136 of *Aceria granati* (54% coverage, 81.65% identity)/YP_009351807 of *Rhinotergum shaoguanense* (99% coverage, 86.27% identity); for OR756239 of *Calacarus pusillus*—MZ483543 of *Tegolophus celtis* (55% coverage, 78.95% identity)/QSJ03309 of *Aceria salvia* (99% coverage, 90.34% identity); for OR756240 of *Jiangsuacarus* sp.—OR414017 of *Leipothrix convallariae* (98% coverage, 77.86% identity)/BBA66506 of *Aceria chibaensis* (82% coverage, 83.65% identity); for OR756235 of *Viginticus searsiae* n. sp.—MZ483565 of *Tegolophus glochidionis* (53% coverage 79.3% identity)/QSJ03309 of *Aceria salviae* (99% coverage, 89.56% identity).

Remarks. When we blasted our sequences against Calacarini, all found calacarine sequences from GenBank showed notably lower values of identity than those found when we blasted them against Eriophyoidea (see above). We conclude that *Cox1* gene is suboptimal for Blast identification of calacarine sequences considering the current number and quality of the calacarine sequences in GenBank.

Blast searches of *28S* sequences of calacarines from our material revealed the following most similar sequences from GenBank: for OR789154 *Calacarus pusillus* (sorted by % identity)—MZ288927 of *Neoshevtchenkella liquidambaris* 54% coverage, 95.87% identity and KM111063 of *Calacarus carinatus* 57% coverage, 95.81% identity; for OR789152 *C. baviensis* n. sp. (sorted by % identity)—MH796800 of Eriophyidae sp. 60% coverage, 89.83% identity and KM111063 of *Calacarus carinatus* 57% coverage, 89.02% identity; for OR789151 *C. burchelliae* n. sp. (sorted by % identity)—MH796800 of Eriophyidae sp. 59% coverage, 93.61% identity and KM111063 of *Calacarus carinatus* 57% coverage, 85.39% identity; for OR789155 *Jiangsuacarus* sp. (sorted by % identity)—MZ288815 of *Jaranasia* cf. *anamensiae* 53% coverage, 95.22% identity; for OR789156 *Paracalacarus podocarpi* (sorted by % identity)—KM111070 of *Paracalacarus podocarpi* 47% coverage, 99.15% identity; for OR789153 *Viginticus searsiae* n. sp. (sorted by identity and filtered by coverage >55%)—KY921995 *Aculus* sp. 58% coverage, 81.26% identity; ON555692 *Abacarus acutatus* 75% coverage, 79.68% identity and KM111063 of *Calacarus carinatus* 57% coverage, 79.66% identity.

Remarks: blast searches for *28S* sequences MH796800 (Eriophyidae sp.) and MZ288927 (*Neoshevtchenkella liquidambaris*) return as best hits different sequences of *Calacarus carinatus* (88/93% identity, 60/96% coverage, respectively), indicating their possible affinities with Calacarini.

3.2.2. Cox1 and D1D2 28S Analyses

Both analyses produced incompletely resolved trees with many well-supported clades (Figure 11). All sequences of calacarines are scattered in *Cox1* and *28S* trees in five similar large zones (I–V) enriched with sequences of Aceriini and Phyllocoptinae associated with monocots (I), cecidophyines (II), nothopodines (III), acaricalines (IV), or "true" calacarines (V, further—tC). The latter zone contained all our sequences plus some calacarine sequences from GenBank, of which affinity with Calacarini was confirmed (or at least not rejected) by Blast searches (see Section 3.2.1). *Cox1* analysis failed to infer *Viginticus searsiae* sp. n. as a member of tC; however, *28S* analysis unambiguously found that it belongs to this group. In general, the results of our molecular phylogenetic analyses (a) suggested possible monophyly of tC; (b) found a questionable position of *Quadratum* and *Paracalacarus* in Eriophyidae, (c) agreed with Blast searches and confirmed the presence of numerous sequences in GenBank that were wrongly assigned to Calacarini (e.g., some sequences of *Jutarus, Jaranasia*, and *Calacarus*); (d) showed that many erroneous sequences of calacarines belong to clades containing sequences of Nothopodinae or Cecidophyinae—the two taxa that can be easily misidentified if morphology of Calacarini is presented inadequately (see Section 3.1, "Remarks on diagnosis of Calacarini"); and (e) inferred sequences MZ482568 and MZ288927 as members of tC, contrary to their initial assignment to noncalacarine genera *Cecidophyes* and *Neoshevtchenkella*.

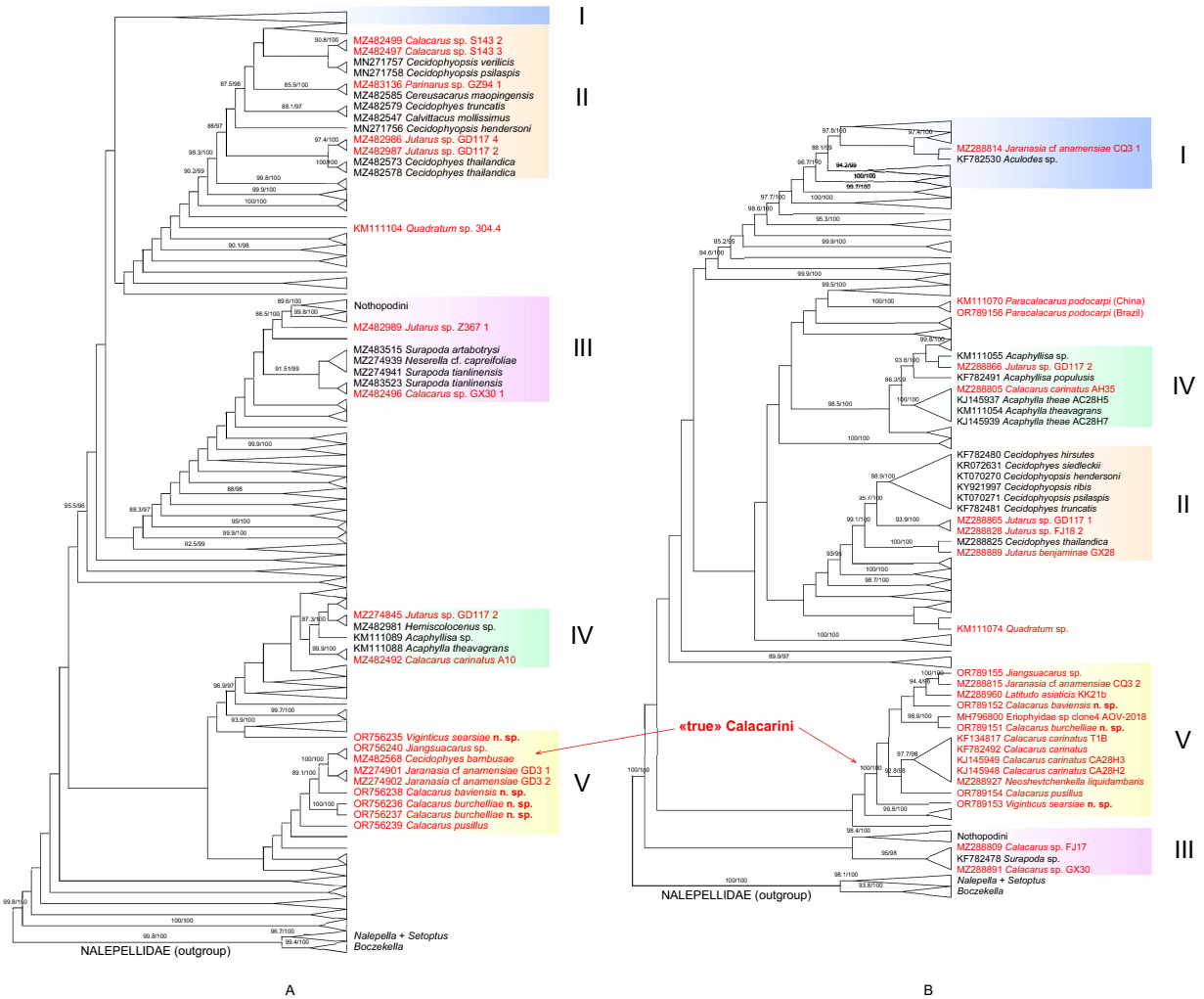

Figure 11. Two maximum likelihood trees (*Cox1*, (**A**) and *28S*, (**B**)) showing relative position of sequences assigned to Calacarini (colored red) in Eriophyidae s.l. Clades without calacarines are collapsed. Five zones enriched with sequences of monocot-associated eriophyids (I, blue), cecidophyines (II, orange), nothopodines (III, purple), acaricalines (IV, green) and "true" calacarines (V, yellow) are indicated. Values of SH-aLRT support (%, >85)/ultrafast bootstrap support (%, >95) are shown above branches.

4. Discussion

"True" and "false" calacarines. This paper contributes to the knowledge on diversity, host-association, and distribution of eriophyoid mites of the tribe Calacarini. It represents new descriptive data on calacarines collected from eudicot (*Burchellia*, *Calluna*, *Mallotus*, *Searsia*), monocot (*Arundinaria*), and evergreen conifer (*Podocarpus*) hosts from five continents. Finding *Calacarus pusillus* Pye on heather (*Calluna vulgaris*) in northwestern Russia is notable because it represents the most northern record of the tribe Calacarini. The findings of *Paracalacarus podocarpi* Keifer in Brazil, *Viginticus searsiae* **n. sp.** in South Africa, and *Jiangsuacarus* sp. in the USA are the first records of the respective mite genera in South America, Africa, and North America. Two of our findings (*Calacarus burchelliae* **n. sp.** in South Africa and *Jiangsuacarus* sp. in the USA) represent new calacarines associations with endemic plants—*Burchellia bubalina* a Southern African endemic [34], and *Arundinaria gigantea,* endemic to central North America [64]. Contrarily, *Calacarus baviensis* **n. sp.** described from Vietnam is associated with the tropical tree *Mallotus paniculatus* that has a very wide distribution including India, China, Southeast Asia and Australia [33].

In this study, we aimed to assess the phylogenetic position of the new species within Eriophyoidea and estimate the phylogeny of the tribe Calacarini based on analyses of *Cox1* and *28S* sequences applying Blast and tree-based approaches. Unexpected complications made this goal difficult to achieve. First, none of the *Cox1* sequences of calacarines from GenBank were inferred to be closest to our *Cox1* sequences based on Blast searches, indicating the low power of the *Cox1* gene for distinguishing calacarines using Blast algorithms. Second, about half of the calacarine sequences from GenBank involved in our analyses were scattered in different non-calacarine lineages of Eriophyidae, suggesting polyphyly of Calacarini (Figure 11). A similar "chaotic" distribution of calacarine sequences is clear in the gene trees presented by previous authors [28,29,63], who interpreted this to be a result of natural nucleotide heterogeneity between mite populations caused by environmental factors. Third, most calacarine sequences that are currently present in GenBank came from two studies [65,66] known to be sources of erroneous sequences [30]. Our tests, focused on validation of the taxonomic identity of calacarine sequences (Section 3.2.1), revealed a new large group of erroneous sequences originating from the works of the same authors and submitted to GenBank. This led us to the opinion that unexpected positions of some calacarine sequences in molecular cladograms reflect their incorrect taxonomic assignment or sample contaminations, which, in turn, impedes testing monophyly of Calacarini.

In our recent paper on the phylogeny of *Leipothrix* [67], we came to similar conclusions and applied epithets "true" and "false" to the clades containing correct and erroneous sequences of *Leipothrix*. Here, we found a stable group of sequences of *Calacarus*, *Jaranasia, Latitudo*, and *Viginticus*, which we call "true calacarines" (tC). Members of these genera are usually purple, produce wax, have tarsal solenidia ω with a large spherical knob, and a net-like pattern of the prodorsal shield, often with a characteristic chain of oval or rectangular cells in the LZ (Figure 2A). Lateral spines or processes of opisthosomal annuli, smooth prodorsal shields, and a median opisthosomal furrow are atypical for tC, as is a reduction of any opisthosomal setae. These traits are common for inadequately described calacarines (e.g., *Lanyuii*, *Taijutarus*, *Quadratum*) or for those that in our analyses clustered with cecidophyines, nothopodines, acaricalines, and monocot-associated eriophyids ("false calacarines" in zones I–IV, Figure 11). Overall, a comprehensive morphological revision of all calacarine taxa is needed, as is validation of taxonomic assignment of all sequences of Calacarini (and other taxa of Eriophyoidea) submitted to GenBank in the last decade. Such revisions could be performed using artificial intelligence programming, a methodology intensively invading all areas of science today [68]. We also predict that recollecting and reinvestigating monotypic calacarine genera from Asia may result in a reduction of the current number of calacarine genera due to new synonymies.

Spermathecal process and genital tubules in Eriophyoidea. In slide-mounted females of Calacarini from our material, we observed paired thin genital tubules (GT) about 10–15 μm long associated with the vestibulum (Figure 5). In the beginning of this study, we

considered them homologs of the paired spermathecal processes (SP), the tiny structures recently discovered in galls mites [69]. Up to now, SP have been described in various taxa of Eriophyidae s.l., whereas in Pentasetacidae and Phytoptidae s.l. they have not been reported [13]. Usually, SP are situated on the postero-medial surface of the spermathecal duct and resemble small thorn-shaped structures about 1 µm long; however, in a few taxa they are slightly longer, e.g., in *Pseudotagmus*, SP is about 3–4 µm long [70]. Our microscopic observations led us to the opinion that GT are separate structures because (a) they join the vestibulum and (b) in all studied specimens we observed tiny SP situated lateral to this junction.

The function of GT and SP is unknown, and they may be rudimentary non-functional elements of the spermathecal apparatus. However, their constant presence in a large set of eriophyoid taxa suggests they may be important for mite reproduction. Although it was shown that females of eriophyoids store sperm cells in spermathecae [71,72], it has never been demonstrated where and at what stage of egg development male and female gametes fuse. It is possible that GT may represent two channels joining the vestibulum with the oviduct and leading the sperm cells towards the oocytes. Alternatively, GT may be the ducts of an unknown female accessory gland still to be discovered, similar to the recently described large silk-producing anal glands which are believed to be present in the caudal part of opisthosoma of all eriophyoid mites [14]. Future research with the aid of various modern microscopy techniques is needed to test these hypotheses and refine our understanding of the anatomy and functioning of the female reproductive system of Eriophyoidea.

5. Conclusions

This paper reports on new species and records of gall mites (Eriophyoidea) of the tribe Calacarini from various endemic and introduced trees from South Africa, Eurasia, and the Americas. It also reveals and resolves some taxonomic problems in Calacarini by revising the morphological concept of several calacarine taxa and proposing a new synonymy. Our study revealed a considerable number of erroneous sequences of gall mites in GenBank that came from a series of large molecular phylogenetic studies performed in China in the last decade. Being included in molecular phylogenetic analyses, these sequences may be the reason for misleading conclusions on the molecular evolution of Eriophyoidea. These sequences wait for their revision and correct taxonomic assignment by the submitters according to the regulatory documentation by GenBank. Our comprehensive investigations of slide-mounted calacarines using advanced Nomarsky (DIC) microscopy resulted in the discovery of paired genital tubules—novel structures of the female reproductive system of Eriophyoidea. We hypothesize them to be the ducts of unknown glands or the channels leading the sperm cells from the spermatheca to the ovarium. These hypotheses are important in terms of the comparative functional anatomy of Acariformes [73] and could be tested using transmission electron microscopy techniques.

Author Contributions: Conceptualization, P.E.C.; methodology, P.E.C. and C.C.; validation, J.A., H.X.T., N.T.T.L., V.D.G. and V.D.N.; formal analysis, P.E.C., C.C., J.A., V.D.G. and V.D.N.; investigation, P.E.C., C.C. and V.D.G.; resources, P.E.C., C.C., H.X.T., N.T.T.L. and V.D.N.; writing—original draft preparation, P.E.C., C.C. and J.A.; writing—review and editing, all coauthors; visualization, P.E.C., C.C., V.D.G. and V.D.N.; supervision, project administration, funding acquisition, P.E.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Ministry of Science and Higher Education of the Russian Federation (agreement 075-15-2022-322 "Agricultural technologies for the Future").

Data Availability Statement: The new sequences of calacarines have been deposited in the National Center for Biotechnology Information (NCBI) GenBank database (https://www.ncbi.nlm.nih.gov/genbank (accessed on 23 November 2023)); accession numbers: OR756235–OR756240, OR789151–OR789156.

Acknowledgments: PCR and sequencing were conducted with the equipment of the "Development of Molecular and Cellular Technologies", "The Bio-Bank", and "Microscopy and Microanalysis" Resource Centers of St. Petersburg State University (Russia). We thank A. Kudryavtzev (St. Petersburg State Agrarian University, Russia) for his assistance in slide-mounting and measuring calacarines. The Galaxy server that was used for some calculations is in part funded by Collaborative Research Centre 992 Medical Epigenetics (DFG grant SFB 992/1 2012) and German Federal Ministry of Education and Research (BMBF grants 031 A538A/A538C RBC, 031L0101B/031L0101C de.NBI-epi, 031L0106 de.STAIR (de.NBI)).

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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