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RESEARCH ARTICLE

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Cerotegument microstructure of whip spiders (Amblypygi: Euamblypygi Weygoldt, 1996) reveals characters for systematics from family to species level

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Abstract

Like other arthropods, whip spiders of the arachnid order Amblypygi Thorell, 1883 protect themselves against external environmental influences. In this taxon, in addition to the epicuticle, the outermost layer of the exoskeleton, a cement layer (cerotegument) with superhydrophobic properties is deposited over certain body parts. Due to the high level of interspecific variation, the cerotegument structure and the morphology of its associated gland openings have been suggested to be informative for whip spider systematics. The first comparative study of the cerotegument is presented herein, based on a survey across 4 families, 16 genera, and 62 species of Euamblypygi Weygoldt, 1996, the suborder comprising all extant whip spiders except the rare monotypic family Paracharontidae Weygoldt, 1996. Results confirmed that the morphology of the colloidal particles and their assembly on cement globules differ considerably among taxa, but that the level of variation differs among lineages. Interspecific variation in cerotegument ultrastructure was highest among species of Neoamblypygi Weygoldt, 1996, making it an informative character in this clade. Evolutionary trends and intraspecific variation in the structure of the amblypygid cerotegument are briefly discussed.

KEYWORDS
Charinidae, Charontidae, exoskeleton, Phrynichidae, Phrynidae, surface coating

1 | INTRODUCTION

The arachnid order Amblypygi Thorell, 1883 (whip spiders) comprises terrestrial predators mainly distributed in the tropics and subtropics, with some exceptions in arid habitats (Harvey, 2003, 2013). Their body plan has remained largely unchanged since the Devonian, about 385 mya (Haug & Haug, 2021). The dorsoventrally flattened body is divided into an anterior prosoma and posterior opisthosoma. The first pair of legs is antenniform, and only three pairs of legs are used for locomotion. Venom glands are absent and prey is captured using the strong, spinose pedipalps (Weygoldt, 2000).

The order currently comprises more than 270 extant species in 17 genera and five families (Miranda et al., 2018, 2021; Weygoldt, 1996, 1999). The little-known family Paracharontidae Weygoldt, 1996 is considered the sister-group of the Euamblypygi Weygoldt, 1996, which is further subdivided into the Charinidae Weygoldt, 1996 and the Neoamblypygi Weygoldt, 1996, which contains the Charontidae Simon, 1892, Phrynichidae Simon, 1900 and Phrynidae Blanchard, 1852 (Weygoldt, 1996, 2000).

Like other arthropods, whip spiders protect themselves against external environmental influences. The outer layer of the exoskeleton, or epicuticle, plays an important role in this regard. However, some arthropods, such as some representatives of

millipedes (Myriapoda Latreille, 1802), mites (Acari Leach, 1817), whip scorpions (Thelyphonida Cambridge, 1872) and whip spiders (Amblypygi) possess an additional outer layer (e.g., Adis et al., 1998; Alberti et al., 1981; Seiter et al., 2021; Wolff et al., 2017), that is, the cerotegument (Maggenti et al., 2005). In whip spiders this cement layer was first observed by Weygoldt (1996) but never described in detail. Recently, two articles by Wolff et al. (2016, 2017) described the ultrastructure, glandular origin, and hydrophobic properties of the whip spider cerotegument. Wolff et al. (2016, 2017) suggested that cerotegument structure and the morphology of the associated gland openings may offer potential characters for whip spider systematics, due to the high levels of interspecific variation observed.

The whip spider cerotegument is secreted after molting and self-assembles into globular structures with a micro-ornamented surface. The fine structure is defined by the specific shape of colloidal particles dispersed in an isotropic matrix, produced by two types of glandular apparatuses (Filippov et al., 2017; Wolff et al., 2016). The ornamentation of the resulting cerotegument structure is hierarchical, as follows: (1) the macrostructure is defined by the shape and arrangement of globules and (2) the fine structure is defined by the diversity, shapes, and arrangement of colloidal nanoparticles (Figure 1). Among Pedipalpi Latreille, 1810, the group including whip spiders and whip scorpions, the general evolutionary trend is toward an increasingly complex cerotegument structure, that is, the number of colloidal nanoparticle types and the complexity of their three-dimensional texture increase (Seiter et al., 2021; Weygoldt, 1996; Wolff et al., 2017).

The cerotegument turns the initially hydrophilic cuticle into a hydrophobic state and leads to the development of a plastron around the body, when submerged in water, that is, during flooding events (Hebets & Chapman, 2000; Wolff et al., 2016). In addition, the cerotegument serves as a growing medium for fungi that are acquired from the environment with unknown function (Gibbons et al., 2019). Fungal hyphae are not to be confused with the cerotegument structures and can usually be easily identified in SEM images by their smooth, collapsed cellular structure.

The present study provides the first comparative study of the cerotegument across the 4 families, 16 genera, and 62 species of Euamblypygi, to assess its potential utility for the systematics of the order.

2 | MATERIAL AND METHODS

2.1 | Taxon sampling and material examined

Sixty-two exemplar species, representing 16 genera and 4 families of Euamblypygi, were studied based on the availability of material. Material from different populations of 13 species was examined to assess the extent of intraspecific variation in cerotegument microstructure. Generally, one individual per species was examined (for details, see Tables 1–5 and supplementary online material). Finally, material from previous studies (Wolff et al., 2016, 2017; Seiter & Wolff, 2017; Seiter et al., 2018, 2021) was re-examined.

Material comprised air-dried exuviae, exuviae preserved in 80% ethanol, or whole specimens preserved in 80% ethanol that were borrowed from the Naturhistorisches Museum Wien (NHMW), Vienna, Austria, and the Staatliches Museum für Naturkunde Stuttgart (SMNS), Stuttgart, Germany.

2.2 | Scanning electron microscopy

Fragments of the cuticle, parts of the carapace or proximal parts of the legs, were excised for scanning electron microscopy (SEM). Fluid preserved samples were completely dehydrated by an increasing ethanol series followed by critical point drying using a Leica CPD300 (Leica Microsystems, Wetzlar, Germany). Dried samples were mounted on standard SEM stubs with double-sided adhesive carbon tape. Dried samples were sputter coated with gold using a Leica EM SCD050. Coated samples were analyzed with a Zeiss Supra 55 VP SEM (Jena, Germany).

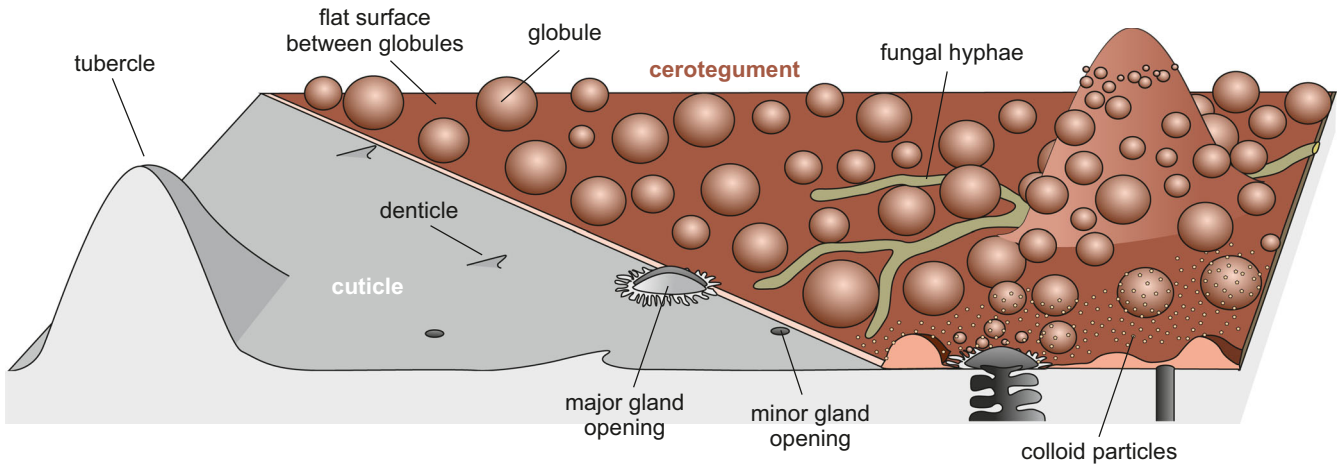

FIGURE 1 Schematic illustration of the cuticular surface of whip-spiders: At left, cuticula only; at right, with cerotegument and mycobiome (i.e., typical inter-molt state)

TABLE 1 Microstructural characteristics of cerotegument in whip spider family Charinidae Quintero, 1986. New = new data; ? = not observed

Species	Origin	Globule surface	Colloid particles	Gland openings	Reference
Charinus acosta (Quintero, 1983)	San Antonio de los Baños, Cuba	Particle network with nanopores	Incomplete cylindrical particles building networks	?	Wolff et al. (2017)
Charinus bahoruco Teruel, 2016 (Figure 2d–f)	Pedernales, Dominican Republic	Particle network with nanopores	Incomplete cylindrical particles building networks and larger spherical particles on top	?	Seiter et al. (2018), new
Charinus cubensis (Quintero, 1983) (Figure 2j–l)	Guantánamo, Cuba	Particle network with nanopores	Fibers and crystals	?	Wolff et al. (2017), new
Charinus dominicanus Armas & Pérez, 2001	Barahona, Dominican Republic	Particle network with nanopores	?	?	Seiter et al. (2018)
Charinus iuiu Vasconcelos & Ferreira, 2016 (Figure 2s–u)	Bahia, Brazil	Regular honeycomb network with nanopores	Crystals assembling in honeycomb network	?	New
Charinus magua Seiteret al., 2018 (Figure 2a–c)	Monseñor Nouel, Dominican Republic	Particle network with nanopores	Smooth surface with nano-pores	?	Seiter et al. (2018), new
Charinus neocaledonicus Kraepelin, 1895 (Figure 2g–i)	North Province, New Caledonia	Particle network with nanopores	Complete cylindrical particles building networks	Radial slits (wrinkled)	Wolff et al. (2017), new
Charinus pescotti Dunn, 1949 (Figure 2m–o)	Queensland, Australia	Fine granular with nanopores and connecting fibers between globules and base	Fibers and crystals	?	Gibbons et al. (2019), new
Sarax brachydactylus Simon, 1892	Metro Manila, Luzon, Philippines	Nubby, granular network and connecting fibers base	Fibers, crystals and globules	?	Wolff et al. (2017)
Sarax curioi Giupponi & Miranda, 2012	Aklan, Panay, Philippines	Granular network and connecting fibers between globules and to base	Fibers and crystals	?	Wolff et al. (2017), Seiter & Wolff (2017)
Sarax ioanniticus (Kritscher, 1959) (Figure 2p–r, 9m)	Rhodos, Greece	Regular nubby and connecting fibers between globules and base	Fibers and crystals	Radial slits (wrinkled)	New
Weygoldtia davidovi (Fage, 1946) (Figure 2v–x)	Nakhon Phanom, Laos	Particle network with nanopores	Fibers and crystals (honeycomb network)	?	New

TABLE 2 Microstructural characteristics of cerotegument in whip spider family Charontidae Simon, 1900. New = new data; ? = not observed

Species	Origin	Globule surface	Colloid particles	Gland openings	Reference
Catageus dammermani (Roewer, 1928) (Figures 3d–f and 9p)	Jawa Barat, Java, Indonesia	Fine nubby and connecting fibers to base	Fibers	Radial slits (wrinkled)	New
Catageus longispina (Gravely, 1915) (Figure 3a–c)	Pahang, Malaysia	Coarse nubby	Smooth surface	?	New
Catageus orientalis (Seiter & Wolff, 2017)	Central Sulawesi, Indonesia	Coarse nubby and connecting fibers to base	Fibers and crystals	Single slit with bulging brim	Seiter & Wolff (2017), Wolff et al. (2017)
Catageus sp. Khao Sok (Figure 3g–i)	Surat Thani, Thailand	Coarse nubby	Fibers and crystals	?	New
Catageus sp. Khao Ya (Figure 3j–l)	Phatthalung, Thailand	Fine nubby and connecting fibers to base	Fibers and crystals	?	New
Charon grayi (Gervais, 1842) (Figure 3p–r)	Luzon, Cebu, Negros Island, Philippines	Fine to coarse nubby	Globules	Bipartite valve, wrinkled brim	Seiter & Wolff (2017), Wolff et al. (2017)
Charon grayi (Gervais, 1842) (Figure 3m–o)	Mindanao Island, Philippines	Fine nubby	Globules	?	New

Note: *Catageus orientalis* (Seiter & Wolff, 2017) was referred to as *Stygophrynus* sp. by Wolff et al. (2017) and described as *Stygophrynus orientalis* Seiter & Wolff, 2017.

3 | RESULTS

3.1 | Charinidae

The three genera of Charinidae were usually distinguishable from one another by the shape of the globular surface and its connection with colloidal particles. However, cerotegument structure was less variable in this family than among Neoamblypygi, and congeners were often indistinguishable. Species of *Charinus* and *Weygoldtia* (Figure 2; Table 1) possessed regular globules assembled by filamentous particles, forming irregular nanopores between them, for example, in *Charinus acosta*, *Charinus bahoruco* (Figure 2e), *Charinus cubensis* (Figure 2j,k), *Charinus dominicanus*, *Charinus magua* (Figure 2b), *Charinus neocaledonicus* (Figure 2g,h), *Charinus pescotti* (Figure 2n), and *Weygoldtia davidovi* (Figure 2w). The cerotegument structure presented a coarse granular network in *Charinus iuiu* (Figure 2t) and the interconnection of the fibrils between the globule and its base was pronounced in *C. pescotti* (Figure 2m). The cerotegument surface between globules usually comprised cylindrical colloidal particles assembled into fibrillar patterns forming networks enclosing nanopores of various sizes (e.g., Figure 2c,f,i,l,o; Table 1). Aside from this general pattern, two modifications were observed. First, in *C. iuiu* (Figure 2s,u) and *W. davidovi* (Figure 2v,x), a more regular honeycomb structure was evident between the globules, and second, in *C. bahoruco* (Figure 2d,f), a slightly larger, spherical type of colloidal particle was assembled on top of the network structure.

The globules possessed a granular surface and an interconnection of fibrils that was evident between the globule and its base in exemplar species of the genus *Sarax* (Figure 2; Table 1), a character shared only with *C. pescotti*, but absent in all other charinid species. A second type of spherical globule, dispersed over the globular surfaces was observed in *Sarax brachydactylus*, but absent in *Sarax curioi* and *Sarax ioanniticus*. The globules of *S. ioanniticus* exhibited regular nubby surfaces (Figure 2q). The cerotegument surfaces between globules consisted of fibrils and crystals interconnected with larger strands in *S. curioi* and *S. ioanniticus* (Figure 2p,r), with additional spherical particles (globules) in *S. brachydactylus*.

Major gland openings were observed in only two species (Table 1): *C. neocaledonicus* and *S. ioanniticus* (Figure 9o), both exhibiting irregularly radially arranged wrinkled slits, covered partly by the cerotegument.

3.2 | Charontidae

The genera of Charontidae were mostly distinguishable from each other, and partly shared some characters with Charinidae. All species exhibited regular, spherical globules with a regular nubby surface (Figure 3; Table 2), but a different degree of grain shape, ranging from fine nubby, for example, in *Catageus* sp. Khao Ya (Figure 3k), *Catageus dammermani* (Figure 3e), and *Charon grayi* from Mindanao, Philippines (Figure 3n) to coarse nubby, for example, in *Catageus* sp. Khao Sok (Figure 3h), *Catageus longispina* (Figure 3b), *Catageus orientalis*, and *C. grayi* from the Philippine islands of Cebu, Luzon, and Negros (Figure 3q). The interconnection of fibrils between the globule and its base was pronounced in all species of *Catageus* genus except for

TABLE 3 Microstructural characteristics of cerotegument in whip spider family Phrynichidae Simon, 1900. New = new data; ? = not observed

Species	Origin	Globule surface	Colloid particles	Gland openings	Reference
Damon annulatipes (Wood, 1869)	KwaZulu-Natal Province, South Africa	Wrinkled nubby, occasionally with dimples	Globules	?	Wolff et al. (2017)
Damon johnstonii (Pocock, 1894) (Figure 4a–c)	South-West Province, Cameroon	Nubby with dimples	Globules, partly clumping together	?	new
Damon medius (Herbst, 1797) (Figure 4d–g)	Senegal-Gambia mix (see Weygoldt, 1999); Benin; Akwa Ibom, Nigeria,	Nubby, occasionally with dimples	Globules	Bipartite valve, smooth brim	Wolff et al. (2017), new
Damon sp. Korup (Figures 4h–j, 9n)	South-West Province, Cameroon	Wrinkled nubby, occasionally with dimples	Dense-crowded globules	Bipartite valve, smooth brim	new
Damon tibialis (Simon, 1876) (Figure 4k–m)	Sao Tomè & Principe	Nubby with dimples	Globules, partly clumping together	?	new
Damon variegatus C. L. Koch, 1850 (Figure 4n–p)	Limpopo Province, South Africa	Wrinkled nubby (clumped together), occasionally with dimples	Globules	?	new
Euphrynichus amanica (Werner, 1936) (Figure 4q–s)	Dodoma, Tanzania	Nubby, without dimples	Globules	?	new
Euphrynichus bacillifer (Gerstaecker, 1873)	"Eastern Africa"	Nubby, without dimples	Globules	Bipartite valve, smooth brim	Wolff et al. (2017)
Musicodamon atlanteus Fage, 1939	Souss-Massa, Morocco	Wrinkled nubby, without dimples	Globules	Bipartite valve, smooth brim	Wolff et al. (2017)
Phrynichodamon scullyi Weygoldt, 1996 (Figure 5m–o)	Western Cape Province, South Africa	Nubby, without dimples	Globules	?	new
Phrynichus ceylonicus (C. L. Koch, 1843)	Sabaragamuwa, Sri Lanka	Nubby, without dimples	Globules	?	Wolff et al. (2017)
Phrynichus deflersi arabicus Weygoldt, 2003 (Figure 4w–y)	Mekka Province, Saudi Arabia	Nubby, occasionally with dimples	Globules	?	Wolff et al. (2017), new
Phrynichus dhofarensis Weygoldt et al., 2002 (Figure 5a–c)	Dhofar, Oman	Nubby, occasionally with dimples	Globules	?	Wolff et al. (2017), new
Phrynichus exophthalmus Whittick, 1940 (Figures 5d–f, 9o)	South-West, Cameroon; "Central Africa" (NHMW 1430)	Nubby, occasionally with dimples	Globules	Bipartite valve, nearly smooth brim	New
Phrynichus jayakari Pocock, 1894 (Figure 4t–v)	Dschanub asch-Scharqiyya, Oman	Nubby, occasionally with dimples	Globules	?	New
Phrynichus orientalis Weygoldt, 1998 (Figure 5g–i)	Kampot, Cambodia; Trang, Thailand	Nubby, without dimples	Globules	?	New
Trichodamon princeps Mello-Leitão, 1935 (Figure 5j–l)	Bahia, Brazil	Nubby, without dimples	Globules	?	New
Xerophrynus machadoi Weygoldt, 1996 (Figure 5p–q)	Erongo, Namibia	Nubby (few, scattered)	Thin isotropic layer	Bipartite valve, without brim	New

Note: *Phrynichus dhofarensis* Weygoldt et al., 2002 was misidentified as *Phrynichus jayakari* Pocock, 1894 by Wolff et al. (2017).

TABLE 4 Microstructural characteristics of cerotegument in whip spider family Phrynidae Blanchard, 1852: Genera *Acanthophrynus* Kraepelin, 1899, *Heterophrynus* Pocock, 1894, and *Paraphrynus* Moreno, 1940. New = new data; ? = not observed

Species	Origin	Globule surface	Colloid particles	Gland openings	Reference
Acanthophrynus coronatus (Butler, 1873)	Jalisco, Mexico	Slightly wrinkled, rather smooth	Crystals, rather smooth	Bipartite valve, smooth brim	Wolff et al. (2017)
Heterophrynus aff. *batesii* (Figures 6a–d, 9l)	Loreto, Peru	Wrinkled, rather smooth or corrugated (two different populations)	Large crystals, connected	Bipartite valve, smooth brim	New
Heterophrynus javieri Seiter & Gredler, 2020	Meta, Colombia	Slightly wrinkled, rather smooth	Crystals, rather smooth	?	Wolff et al. (2017)
Paraphrynus carolynae Armas, 2012	Arizona, U.S.A.	Wrinkled	Large crystals	Valve of multiple radially arranged parts	Wolff et al. (2017)
Paraphrynus cubensis Quintero, 1983	San Antonio de los Baños, Cuba	Particle network with pores	Small crystals, connected	?	Wolff et al. (2017)
Paraphrynus laevifrons (Pocock, 1894) (Figures 6e–h, 9k)	Panamá, Panama; Coclé, Panama	Moderately wrinkled, with smooth bottom	Lamella-like, sharp crystals	Bipartite valve, smooth surface, nearly smooth brim	New
Paraphrynus pseudomexicanus Seiter et al., 2020	Morelos, Mexico	Particle network with pores	Small crystals, connected	?	Wolff et al. (2017)
Paraphrynus raptator (Pocock, 1902) (Figure 6l–n)	Cayo District, Belize	Wrinkled	Small crystals	?	New
Paraphrynus robustus (Franganillo, 1931)	Guantánamo, Cuba	Wrinkled	Sharp crystals, connected	?	Wolff et al. (2017)
Paraphrynus sp. Veracruz (Figure 6i–k)	Veracruz, Mexico	Dense particle network with dimples	Small crystals with pores, connected	?	New
Paraphrynus viridiceps (Pocock, 1894)	Cienfuegos, Cuba	Wrinkled	Sharp crystals, connected	Bipartite valve, slightly wrinkled brim	Wolff et al. (2017)

Note: *Heterophrynus javieri* Seiter & Gredler, 2020 was referred to as *Heterophrynus* sp. by Wolff et al. (2017); *Paraphrynus pseudomexicanus* Seiter et al., 2020 was referred to as *Paraphrynus* sp. by Wolff et al. (2017).

Catageus sp. Khao Sok (Figure 3g) and probably *C. longispina* (Figure 3a). This interconnection was absent in all populations of *Charon*. The cerotegument surface between the globules was covered with colloidal particles forming fibrillar and crystalline nanoparticles, for example, in *Catageus* sp. Khao Sok (Figure 3g,i), *Catageus* sp. Khao Ya (Figure 3j,l), and *C. orientalis*, or only fibrils, for example, in *C. dammermani* (Figure 3d,f) and globules, for example, in *C. grayi* (Figure 3m,o,p,r). The underlying surface was smooth, but with poorly expressed nanopores (cf. Figure 2a,c), in *C. longispina* (Figure 3a,c), as in *Charinus magua*.

The colloidal particles of all species of *Catageus*, except *C. longispina*, resembled those of *Sarax*, in comprising two types, fibrillar and spherical. In contrast, the fibrillar particles were absent and the overall surface more regular in *C. grayi*. Major gland openings were only observed in three species, that is, *C. dammermani*, *C. grayi*, and *C. orientalis* (Figure 9p). *Catageus orientalis* possessed single slit gland openings with bulging brims.

The gland openings of *C. grayi* exhibited a bipartite valve with a wrinkled brim (Wolff et al., 2017). The gland openings of *C. dammermani* comprised radially arranged, wrinkled slits (Figure 9p).

3.3 | Phrynichidae

Most of the genera of Phrynichidae were distinguishable from one another based on the shape of the globule surface and the connection of the colloidal particles. All phrynichid species except *Xerophrynus machadoi* (Figure 5p–q) exhibited regularly shaped globules with a nubby surface (Figures 4 and 5; Table 3). The nubby texture was observed in all exemplar species, except *Damon annulatipes*, *Damon* sp. Korup (Figure 4h,i), *Damon variegatus* (Figure 4n,o), and *Musicodamon atlanteus*, in which the texture was wrinkled. A more (without) (Figures 4q,r and 5g,j,m,n) or less (with) (Figures 4a,b,d,e,g, k,l,n,p,t,u,w,x and 5a,b,d,e) dimpled surface was evident in some species, due to a different degree of regularity in the distribution of colloidal particles. Dimples were absent or indistinct in *Euphrynichus amanica* (Figure 4r), *Euphrynichus bacillifer*, *M. atlanteus*, *Phrynichus ceylonicus*, *Phrynichus orientalis* (Figure 5h), *Trichodamon princeps* (Figure 5k), and *X. machadoi* (Figure 5p–q). The remaining phrynichid exemplar species occasionally exhibited dimples across the entire globule surface. No fibrils connecting the globule and the base were observed among the phrynichid exemplar species. Cerotegument surfaces between globules were covered with somewhat regularly

TABLE 5 Microstructural characteristics of cerotegument in whip spider family Phrynidae Blanchard, 1852: Genus *Phrynus* Lamarck, 1801. New = new data; ? = not observed

Species	Origin	Globule surface	Colloid particles	Gland openings	Reference
Phrynus barbadensis (Pocock, 1894)	Aruba, Netherlands Antilles; Barbados	Wrinkled	Crystals	Bipartite valve, slightly wrinkled brim	Wolff et al. (2017)
Phrynus damonidaensis Quintero, 1981 (Figure 9a)	Santiago de Cuba, Cuba	Irregular honeycombed, nubby	Crystals	Bipartite valve, smooth brim	Wolff et al. (2017), new
Phrynus decoratus Teruel & Armas, 2005 (Figures 7p–r, 9j)	Cienfuegos, Cuba; Independencia, Dominican Republic	Honeycombed, more plain than nubby (Dominican population) or honeycombed, nubby (Cuban population)	Continuous honeycombed (Dominican population) or nubby (Cuban population)	Bipartite valve, slightly wrinkled brim (Cuban population)	Wolff et al. (2017), Seiter et al. (2021), new
Phrynus eucharis Armas & Peréz, 2002 (Figures 8a–g, 9e–f)	Puerto Plata, La Altagracia, San Pedro de Macorís, Dominican Republic	Wrinkled to with dimples (Puerto Plata Province) or particle network with nanopores	Small dense crystals (all populations)	Bipartite valve, slightly wrinkled brim (Puerto Plata), or bipartite valve, covered with a particle network with nanopores (San Pedro de Macorís)	New
Phrynus exsul Harvey, 2002	East-Nusa-Tenggara, Indonesia	Wrinkled	Crystals	?	Wolff et al. (2017)
Phrynus goesii Thorell, 1889 (Figure 9b)	St. Maartin (Lesser Antilles); Guadeloupe	Wrinkled	Crystals	Bipartite valve, smooth brim	Wolff et al. (2017), new
Phrynus hispaniolae Armas & Peréz, 2002 (Figure 7m–o)	San Cristóbal, Dominican Republic	Wrinkled with dimples	Small dense crystals	?	New
Phrynus kennidae Armas & Peréz, 2002 (Figures 7s–y, 9i)	Pedernales, Dominican Republic	Particle network with pores, partly honeycombed	Small dense crystals	Bipartite valve, covered with a particle network with pores, wrinkled brim	New
Phrynus longipes (Pocock, 1894) (Figure 9c)	La Altagracia, Samana, Monseñor Nouel, Dominican Republic	Wrinkled without dimples or wrinkled with dimples	Crystals (all populations)	Bipartite valve, wrinkled brim	Wolff et al. (2017), new
Phrynus maesi Armas, 1995 (Figure 8h–j)	León, Nicaragua	Wrinkled with dimples	Large, wrinkled crystals	?	New
Phrynus marginemaculatus C.L. Koch, 1840 (Figures 7a–l, 9g–h)	Baoruco, Puerto Plata, Pedernales, Dominican Republic	Honeycombed, nubby or coralline with nanopores, nubby	Small crystals, partly connected (all populations)	Bipartite valve, wrinkled brim or bipartite valve, slightly wrinkled brim, covered with coralline-like colloid particles	Wolff et al. (2017), new
Phrynus parvulus Pocock, 1902	Cayo District, Belize	Wrinkled with connecting fibers between globules	Crystals	?	Wolff et al. (2017)
Phrynus pinarensis Franganillo, 1930	Pinar del Río, Cuba	Wrinkled with dimples	Crystals	?	Wolff et al. (2017)
Phrynus sp. Dominican Republic (Figure 7j–l)	Ovideo, Pedernales, Dominican Republic	Coralline or wrinkled with dimples	Small crystals, partly connected	?	New
Phrynus sp. Dominican Republic	Monseñor Nouel, Dominican Republic	Wrinkled, rather corrugated	Lamella-like, sharp crystals	?	New
Phrynus whitei Gervais, 1842 (Figures 8k–m, 9d)	Atlántida, Honduras	Wrinkled with dimples	Lamella-like crystals	Bipartite valve, covered with lamelliform crystals, smooth brim	New

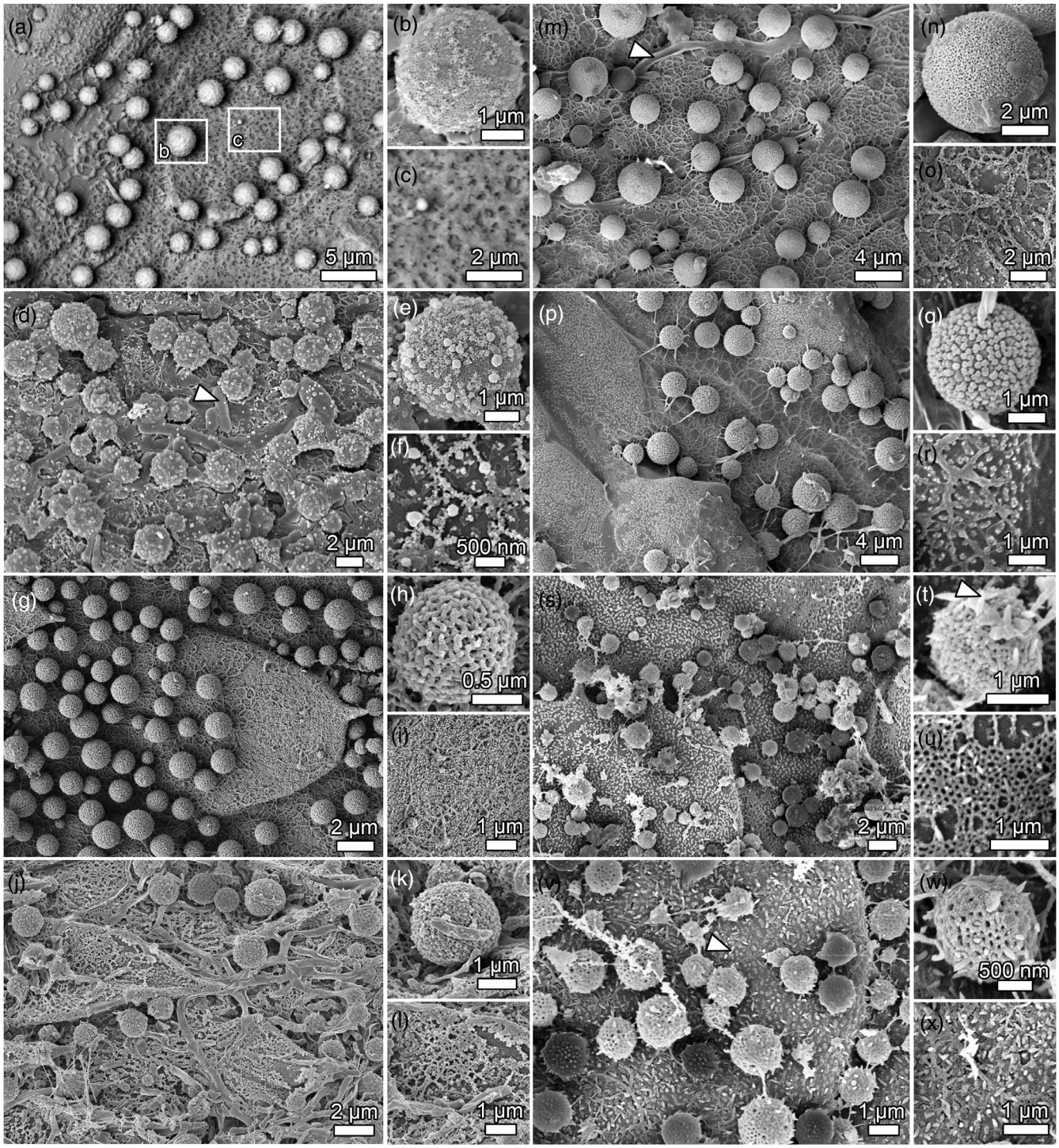

FIGURE 2 Scanning electron micrographs illustrating cerotegument of whip spiders in family Charinidae Quintero, 1986 from air dried carapace exuvia (a–b, g–r) and critical point dried walking leg and part of carapace from ethanol-preserved specimen (d–f, s–x): Overview of surface (left), detail of globule surface and structure (upper right), and detail of colloidal particles (lower right) (a–c) *Charinus magua* Seiter et al., 2018. (d–f) *Charinus bahoruco* Teruel, 2016. (g–i) *Charinus neocaledonicus* Kraepelin, 1895. (j–l) *Charinus cubensis* (Quintero, 1983). (m–o) *Charinus pescotti* Dunn, 1949. (p–r) *Sarax ioanniticus* (Kritscher, 1959). (s–u) *Charinus iuiu* Vasconcelos & Ferreira, 2016. (v–x) *Weygoldtia davidovi* (Fage, 1946). Arrowheads show different contaminants and foreign particles: Fungal hyphae (d, m), and needle-like particles (t, v). Inset in (a) illustrates positions of magnified images of globule surface (b) and colloidal particles (c)

spaced spherical colloidal nanoparticles (e.g., Figures 4f,q,v,y and 5c,f,i,l, o) but were occasionally clumped together, for example, in *Damon johnstonii* (Figure 4c) and *Damon tibialis* (Figure 4m) or densely clustered, for example, in *Damon* sp. Korup (Figure 4j). The cerotegument of *X. machadoi* was highly reduced with only a thin isotropic layer remaining and only a few nubby globules evident (Figure 5p–q).

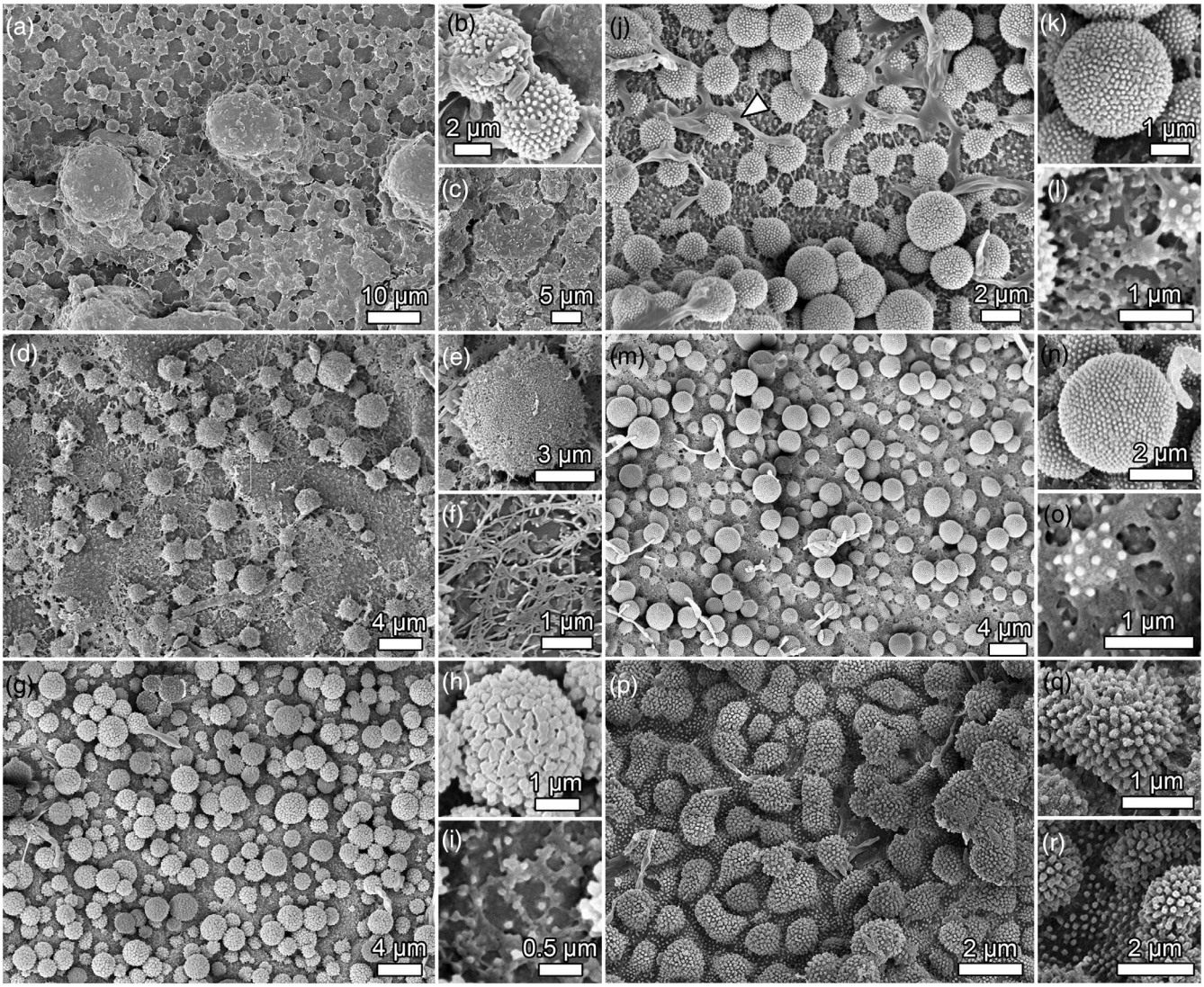

FIGURE 3 Scanning electron micrographs illustrating cerotegument of whip spiders in family Charontidae Simon, 1900 from air dried carapace exuvia (a–c, g–r) and critical point dried walking leg and part of carapace from ethanol-preserved specimen (d–f): Overview of surface (left), detail of globule surface and structure (upper right), and detail of colloidal particles (lower right). For details and interpretation, refer to the results section. (a–c) *Catageus longispina* (Gravely, 1915). (d–f) *Catageus dammermani* (Roewer, 1928). (g–i) *Catageus* sp., Khao Sok, Thailand. (j–l) *Catageus* sp., Khao Ya, Thailand. (m–o) *Charon grayi* (Gervais, 1842), Mindanao, Philippines. (p–r) *C. grayi*, Cebu, Philippines

Where observed, the major gland openings were represented by bipartite valves with a relatively smooth brim, for example, in *Damon* sp. Korup (Figure 9n) and *Phrynichus exophthalmus* (Figure 9m), or without a specific brim, for example, in *X. machadoi* (Figure 5q insert).

3.4 | Phrynidae

The four genera of Phrynidae may be distinguished by various characters of the thick cerotegument layer. Members of the genera *Acanthophrynus*, *Heterophrynus*, and *Paraphrynus* exhibited regularly shaped globules with an underlying surface of small crystalline colloidal nanoparticles (Figure 6; Table 4). The globular surface was observed to be wrinkled, as in *Acanthophrynus coronatus*, *Heterophrynus javieri*, *Heterophrynus* aff. *batesii* (Figure 6a,b,d), and the species of *Paraphrynus* (Figure 6e,f,h,l,m), or formed by a particle network with pores or small dimples, as in *Paraphrynus cubensis* and *Paraphrynus pseudomexicanus*, or with deep dimples, as in *Paraphrynus* sp. Veracruz (Figure 6i, j). The cerotegument surface between the globules was covered with colloidal nanoparticles in the shape of smooth crystals, as in *A. coronatus* and *H. javieri*, or connected small crystals with pores, as in *Paraphrynus* sp. Veracruz (Figure 6k). The colloidal nanoparticles were represented by confluent small to large crystals in other exemplar species, for example, in *Heterophrynus* aff.

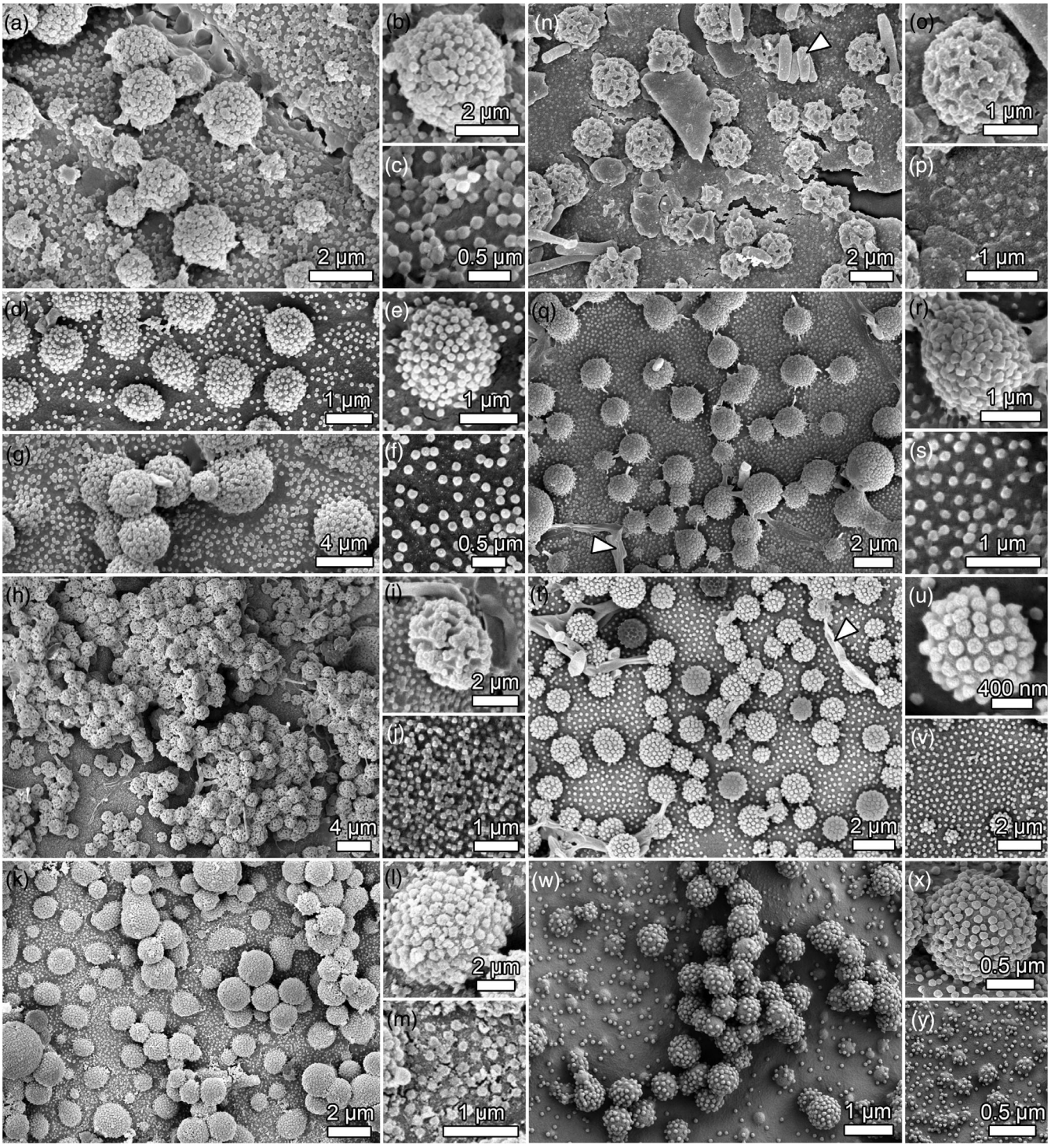

FIGURE 4 Scanning electron micrographs illustrating cerotegument of whip spiders in family Phrynichidae Simon, 1900 from air dried carapace exuvia (a–j, q–y) and critical point dried walking leg from ethanol-preserved specimen (k–p): Overview of surface (left), detail of globule surface and structure (upper right), and detail of colloidal particles (lower right). (a–c) *Damon johnstonii* Pocock, 1894, (Pocock, 1894). (d–f) *Damon medius* (Herbst, 1797), Benin. (g) *Damon medius*, Nigeria. (h–j) *Damon* sp. Korup, Cameroon. (k–m) *Damon tibialis* (Simon, 1876). (n–p) *Damon variegatus* (Perty, 1834). (q–s) *Euphrynichus amanica* (Werner, 1916). (t–v) *Phrynichus jayakari* Pocock, 1894. (w–y) *Phrynichus deflersi arabicus* Weygoldt, 2003

batesii (Figure 6c), *P. cubensis*, and *P. pseudomexicanus*, by confluent sharp crystals, for example, in *Paraphrynus robustus* and *Paraphrynus viridiceps*, by lamelliform sharp crystalline networks, for example, in *Paraphrynus laevifrons* (Figure 6g), or by disconnected small to large crystals, for example, in *Paraphrynus raptator* (Figure 6n) and *Paraphrynus carolynae*.

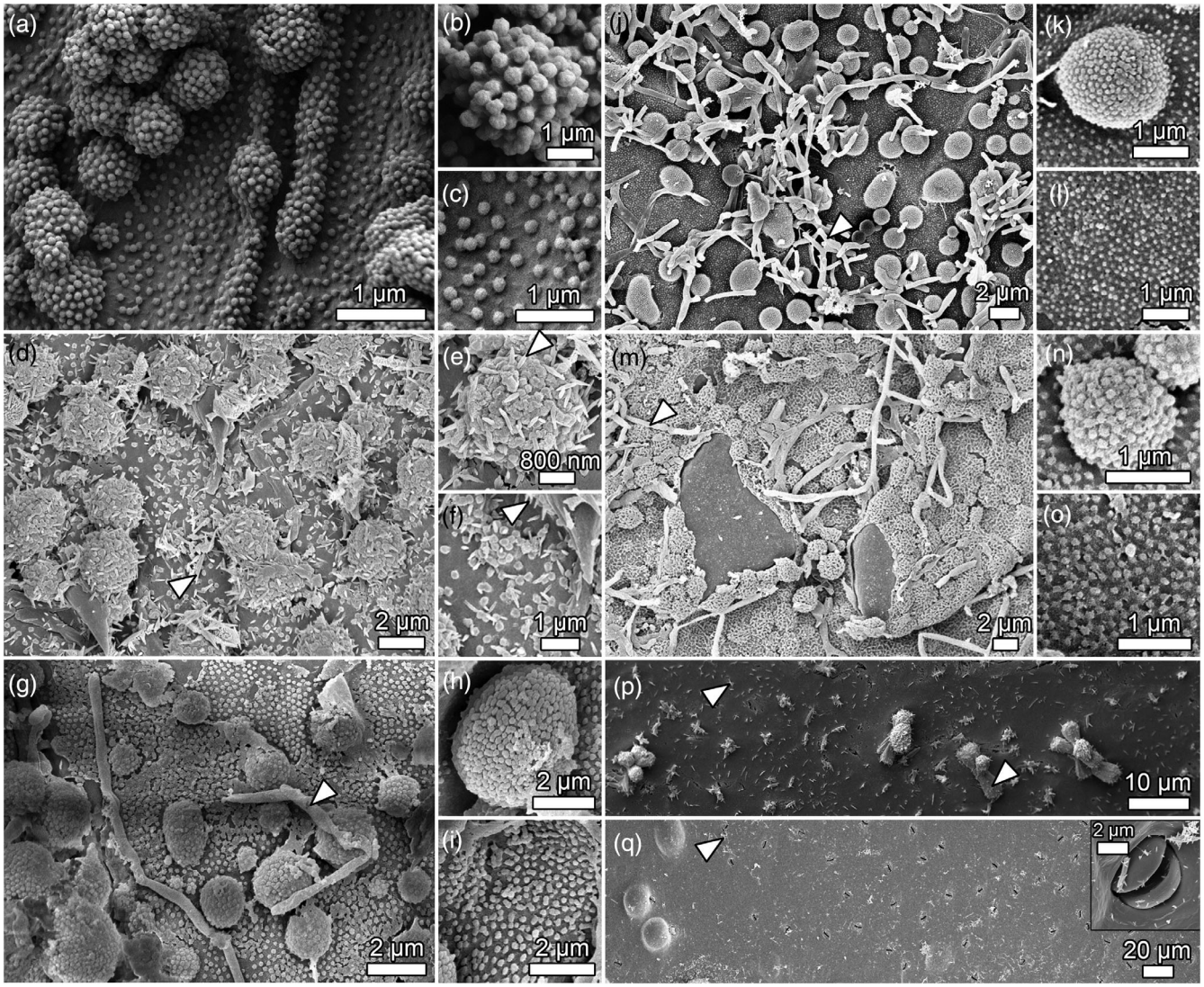

FIGURE 5 Scanning electron micrographs illustrating cerotegument of whip spiders in family Phrynichidae Simon, 1900 from air dried carapace exuvia (a–c) and critical point dried walking leg of ethanol-preserved specimen/exuvia (d–q): Overview of surface (left), detail of globule surface and structure (upper right), and detail of colloidal particles (lower right), except for (p–q), in which only overview of relatively smooth surface (p), area with many major glands (q) and insert with major gland presented. (a–c) *Phrynichus dhofarensis* Weygoldt et al., 2002. (d–f) *Phrynichus exophthalmus* Whittick, 1940. (g–i) *Phrynichus orientalis* Weygoldt, 1998. (j–l) *Trichodamon princeps* Mello-Leitão, 1935. (m–o) *Phrynichodamon scullyi* (Purcell, 1902). (p–q, insert) *Xerophrynus machadoi* Weygoldt, 1996

The greatest diversity of cerotegument structure was observed in the genus *Phrynus* (Figures 7–8; Table 5). Globular surfaces were either wrinkled or wrinkled with dimples, as in *Phrynus barbadensis*, *Phrynus eucharis* (Figure 8a,b), *Phrynus exsul*, *Phrynus goesii*, *Phrynus hispaniolae* (Figure 7m,n), *Phrynus longipes*, *Phrynus maesi* (Figure 8i), *Phrynus pinarensis*, *Phrynus* sp. Monseñor Nouel, and *Phrynus whitei* (Figure 8l). The surfaces were honeycombed with a varying degree of nubbiness, as in *Phrynus damonidaensis*, *Phrynus decoratus* (Figure 7q), and *Phrynus marginemaculatus* from the Dominican Republic, or represented by a particulate network of nanopores, as in *P. eucharis* (Figure 8e) and, to some extent, *Phrynus kennidae* (Figure 7s,t,v,w,x,y), or coralline patterns with nanopores and a nubby subtexture, as in some populations of *P. marginemaculatus* (Figure 7a,b,d,e,g,h). A combination of characters was observed in some species, for example, wrinkles with connecting fibrils between globules and the base (resembling the charinids and *Catageus*), as in *Phrynus parvulus*, or coralline textures with nanopores and wrinkles, as in *Phrynus* sp. Oviedo (Figure 7j,k). The cerotegument surface between globules was covered with colloidal nanoparticles comprised mainly of crystals or small dense crystals, as in *P. eucharis* (Figure 8c,f,l,o), *P. kennidae* (Figure 7u), and *P. marginemaculatus* (Figure 7c,f,i). Other characters included large, wrinkled crystals, observed in *P. maesi* (Figure 8j), lamelliform crystals in *Phrynus* sp. Monseñor Nouel and *P. whitei* (Figure 8m), or a more continuous honeycombed ornamentation in *P. decoratus* (Figure 7r).

The major gland openings of Phrynidae were represented by a bipartite valve with a smooth brim, as in *A. coronatus* and *P. laevifrons*

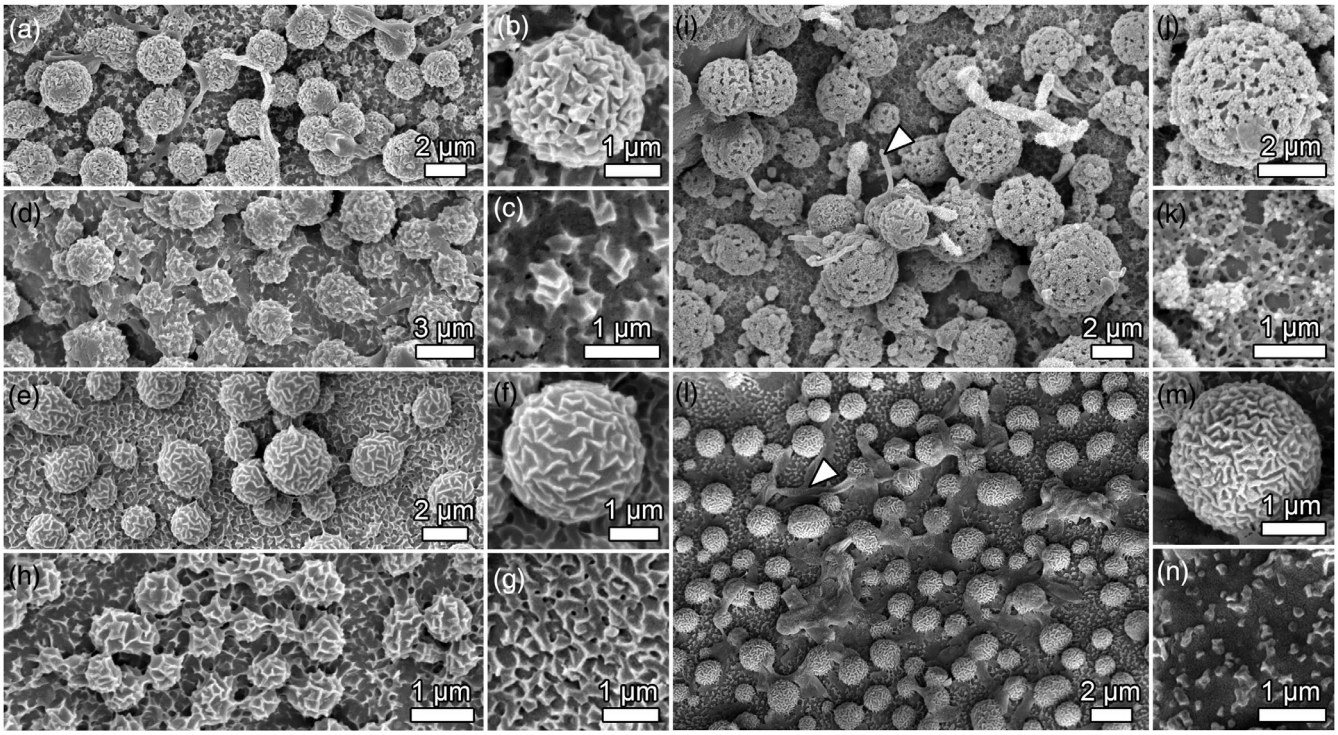

FIGURE 6 Scanning electron micrographs illustrating cerotegument of whip spiders in family Phrynidae Blanchard, 1852 from air dried carapace exuvia: Overview of surface (left), details of globular surface and structure (upper right), and detail of colloidal particles (lower right). (a–c) *Heterophrynus* aff. *batesii*, Tamshiyacu, Peru. (d) *Heterophrynus* aff. *batesii*, Iquitos, Peru. (e–g) *Paraphrynus laevifrons* (Pocock, 1894), Panamá Province, Panama. (h) *Paraphrynus laevifrons*, Coclé Province, Panama. (i–k) *Paraphrynus* sp., Veracruz, Mexico. (l–n) *Paraphrynus raptator* (Pocock, 1902)

(Figure 9k), a bipartite valve with a wrinkled brim, as in *P. viridiceps*, and the species of *Phrynus* (Figure 9c–j), or a valve comprising multiple, radially arranged parts, as in *P. carolynae*.

3.5 | Intraspecific variation

Specimens of the phrynichid, *Damon medius*, from Gambia-Senegal, Benin (Figure 4g) and Nigeria (Figure 4h–f) did not exhibit differences in the microstructure or shape of colloidal nanoparticles (Table 3).

Two populations of the phrynid, *Heterophrynus* aff. *batesii*, from the vicinity of Iquitos, Peru, exhibited a wrinkled globule surface without further nanotexture (Figure 6a–d). However, the globular surface appeared more smooth and less crystalline in one individual (Figure 6d; Table 4), probably an artifact of the sample preparation or the hardening process after molting in this particular individual. A similar observation was made in two different populations of *Paraphrynus laevifrons* from Panama (Figure 6e–h; Table 4). These minor differences were not considered indicative of intraspecific variation.

Similarly, few to no differences were observed among the remaining six phrynid species, in which more than one population was examined. One population of *Phrynus eucharis* exhibited a wrinkled (with dimples) globular surface, whereas the other two populations exhibited a particle network with nanopores (Figure 8a–g; Table 5). However, the general appearance matched other Caribbean phrynids (Table 5). Two populations of *Phrynus decoratus* from Cuba and Hispaniola (Dominican Republic) differed only in minor respects, exhibiting a honeycombed globular surface with a nubby or plain surface (Figure 7p–r; Table 5).

Four populations of the charontid, *Charon grayi*, were analyzed from the Philippine islands of Cebu, Luzon, Mindanao, and Negros. The specific status of these populations remains unclear, and they may represent a species complex (Weygoldt, 2002). However, their cerotegument microstructure did not permit any distinction, as all four populations exhibited a fine to coarse nubby globular surface with spherical colloidal particles (Figure 3m–r; Table 2).

3.6 | Contaminants

Different contaminants and foreign particles, as well as microorganisms, are common on the cerotegument. This is important for the interpretation of SEM images of cerotegument structure. Microorganisms, spores (e.g., Figures 4n and 5p) and fungal hyphae were commonly observed (e.g., Figures 2d,m, 3j, 4q, t, 5g,j,m, 6i,l, and 8h, k). Needle-like, filamentous, or crystalline particles, most likely derived from environmental contaminants and not part of the cerotegument, were also present on occasion (e.g., Figures 2s,t,v and 5d,e,f,p,q).

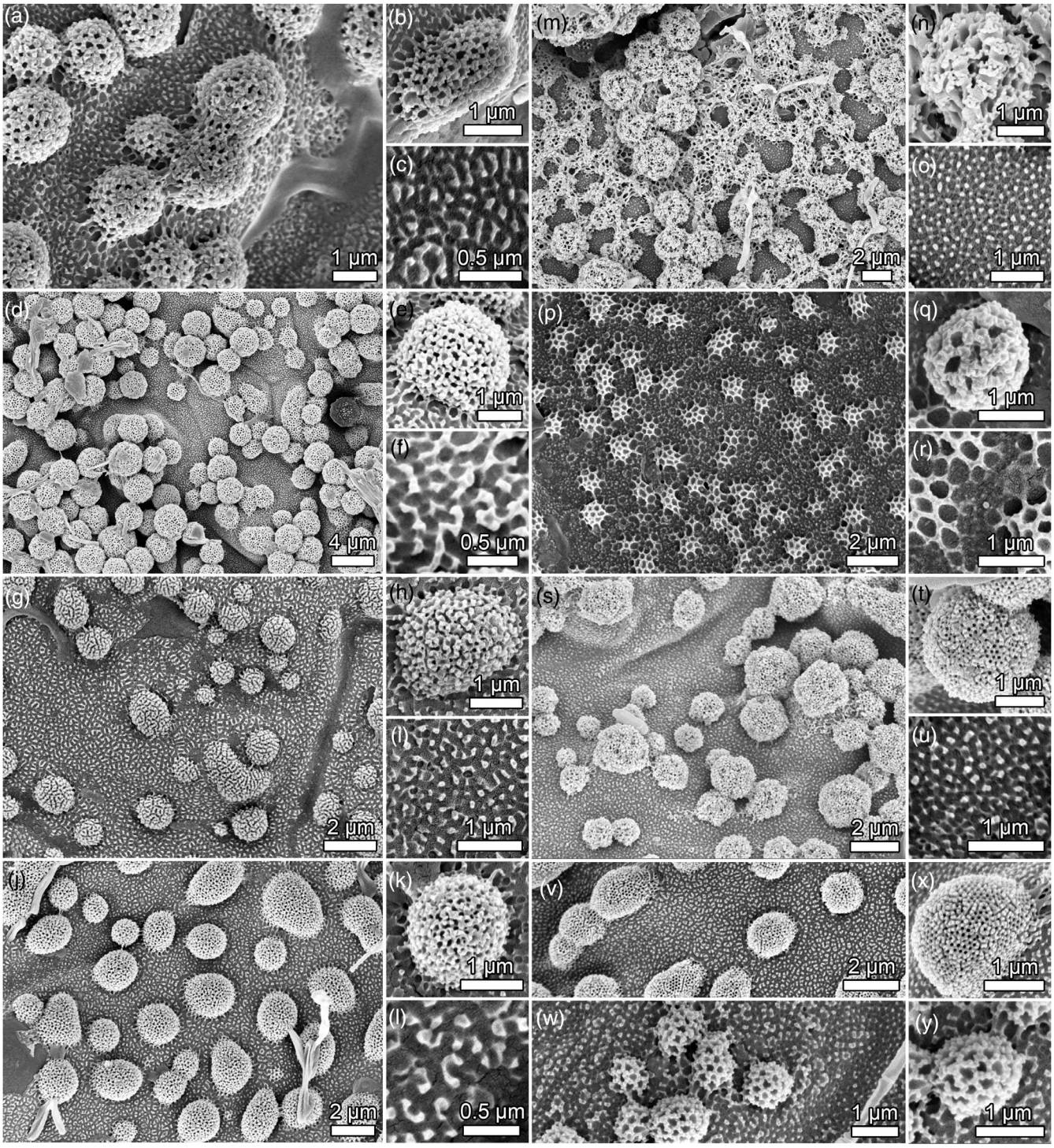

FIGURE 7 Scanning electron micrographs illustrating cerotegument of whip spiders in family Phrynidae Blanchard, 1852 from air dried carapace exuvia: Overview of surface (left), detail of globule surface and structure (upper right), and detail of colloidal particles (lower right), except (y), in which globule surface is shown. (a–c) *Phrynus marginemaculatus* C.L. Koch, 1840, Baoruco Province, Dominican Republic. (d–f) *P. marginemaculatus*, Puerto Plata Province, Dominican Republic. (g–i) *P. marginemaculatus*, Pedernales Province, Dominican Republic. (j–l) *Phrynus* sp., Oviedo Province, Dominican Republic. (m–o) *Phrynus hispaniolae* Armas & Peréz, 2001, San Cristóbal Province, Dominican Republic. (p–r) *Phrynus decoratus* Teruel & Armas, 2005, Barahona Province, Dominican Republic. (s–u) *Phrynus kennidae* Armas & Peréz, 2001, Pedernales Province, Dominican Republic (18°02′59.28″N 71°38′17.791″W). (v–y) *P. kennidae*, Pedernales Province, Dominican Republic (18°04′23.279″N 71°39′09.18″W)

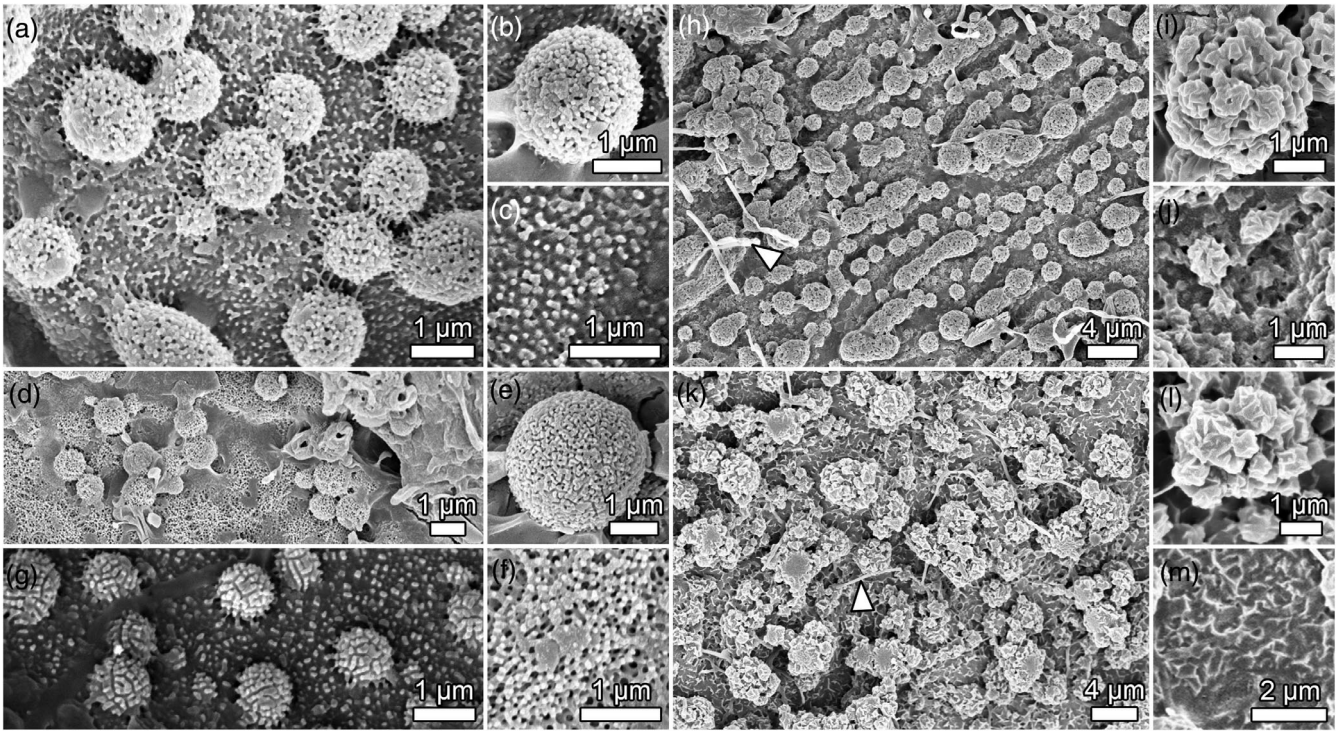

FIGURE 8 Scanning electron micrographs illustrating cerotegument of whip spiders in family Phrynidae Blanchard, 1852 from air dried carapace exuvia: Overview of surface (left), detail of globule surface and structure (upper right), and detail of colloidal particles (lower right). (a–c) *Phrynus eucharis* Armas & Peréz, 2001, San Pedro de Macorís Province, Dominican Republic. (d–f) *P. eucharis*, La Altagracia Province, Dominican Republic. (g) *P. eucharis*, Puerto Plata Province, Dominican Republic. (h–j) *Phrynus maesi* Armas, 1995. (k–m) *Phrynus whitei* Gervais, 1842

4 | DISCUSSION

4.1 | Interspecific variation

Interspecific variation in the cerotegument structure of whip spiders confirms its utility as a character at the family level, and often also at the generic level. In some taxa, the variation may even be useful at the species level.

In Charinidae, the globular surface consisted mainly of a particle network or possessed fine nubby to granular globules, partly with connecting fibrils between globules and the base. Connecting fibrils were specific to some Charinidae, the genus *Catageus* and one phrynid exemplar species, *Phrynus parvulus*. The globular surface was nubby to some extent in all exemplar species of Charontidae.

The hypothesized phylogenetic relationship between Charinidae and Charontidae (Weygoldt, 1996) was supported by the microstructure of the cerotegument. The colloidal particles of Charinidae consisted mostly of fibrils and crystals, partly forming a network. This character was shared with most exemplar species of the charontid genus, *Catageus*, except for *C. longispina*, which exhibited an amorphous smooth layer on the surface, unique among the exemplar species of Euamblypygi and resembling the simple cerotegument structure observed in the order Thelyphonida (Seiter et al., 2021). In contrast, however, the colloidal particles consisted of globules in the other charontid genus, *Charon*. The observation that Charinidae share cerotegument characters with *Catageus*, but not *Charon*, could indicate symplesiomorphy of the character or paraphyly of Charontidae. The phylogenetic placement of *Catageus* as sister to *Charon*, and the monophyly and diagnosis of Charontidae merit further investigation (Seiter & Wolff, 2017; Wolff et al., 2017).

Cerotegument characters shared by all exemplar species of Phrynichidae included a wrinkled nubby or nubby globular surface with or without dimples in between, and spherical colloidal nanoparticles. The only exception was *Xerophrynus machadoi*, in which the globular surface consisted of only a few nubby globules, distributed across the surface, and the underlying colloidal particles were indistinct and formed only a thin layer. Weygoldt (2000) noted that in this species, the granular structure appeared to be less developed or even absent, which was unusual. As this species appears to be the most arid-adapted whip spider, a constant water-repellent surface could be unnecessary, due to the low humidity and infrequent rainfall in its habitat (Weygoldt, 2000; Wolff et al., 2017). However, in other species adapted to arid environments, for example, *Musicodamon atlanteus*, a regular cerotegumental coverage was observed (Wolff et al., 2017). Tests of "wettability" and surface free energy (SFE) should be carried out on living specimens to verify this hypothesis.

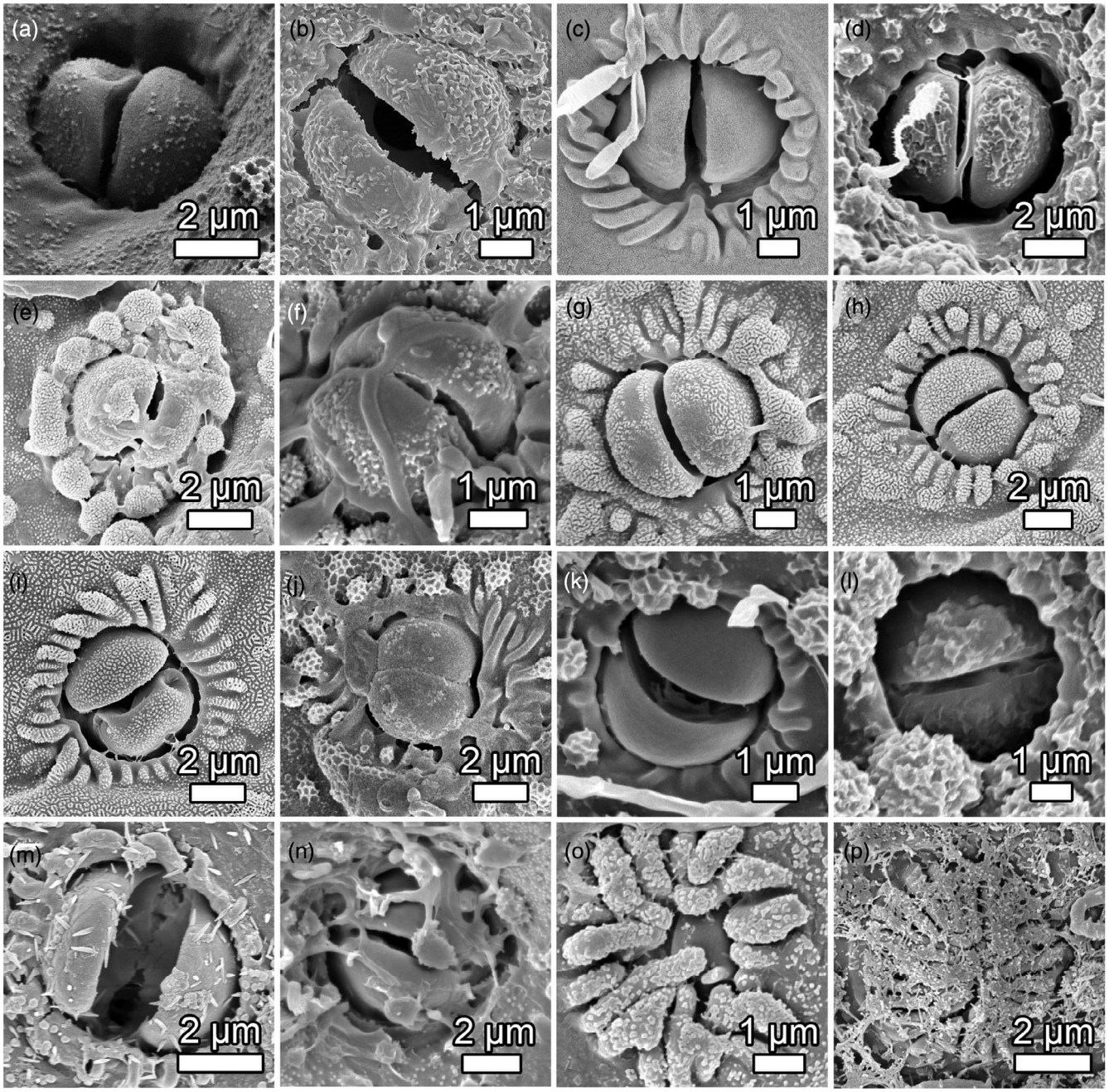

FIGURE 9 Scanning electron micrographs illustrating inter- and intraspecific variation in morphology of major gland openings in whip spiders from air dried carapace exuvia. (a) *Phrynus damonidaensis* Quintero, 1981. (b) *Phrynus goesii* Thorell, 1889. (c) *Phrynus longipes* (Pocock, 1894), Subida a Casabíto, Dominican Republic. (d) *Phrynus whitei* Gervais, 1842. (e) *Phrynus eucharis* Armas & Peréz, 2001, San Pedro de Macorís Province, Dominican Republic. (f) *P. eucharis*, Puerto Plata Province, Dominican Republic. (g) *Phrynus marginemaculatus* C.L. Koch, 1840, Baoruco Province, Dominican Republic. (h) *P. marginemaculatus*, Pedernales Province, Dominican Republic. (i) *Phrynus kennidae* Armas & Peréz, 2001. (j) *Phrynus decoratus* Teruel & Armas, 2005. (k) *Paraphrynus laevifrons* (Pocock, 1894), Coclé Province, Panama. (l) *Heterophrynus* aff. *batesii*, Iquitos, Peru. (m) *Phrynichus exophthalmus* Whittick, 1940. (n) *Damon* sp. Korup, Cameroon. (o) *Sarax ioanniticus* (Kritscher, 1959). (p) *Catageus dammermani* (Roewer, 1928)

Cerotegument microstructures proved useful for species delimitation among four species of *Damon* from West Africa, that is, *D. medius*, *D. johnstonii*, *D. tibialis*, and *Damon* sp. Korup. Although it is presently unknown whether *Damon* sp. Korup is conspecific with *Damon uncinatus* Weygoldt, 1999, or represents an undescribed species, the cerotegument clearly separates this exemplar from the other exemplar species of West Africa, and suggests a closer relationship to the southern African species, *Damon variegatus*.

Similarly, three exemplar species of *Phrynichus*, *P. deflersi arabicus*, *D. dhofarensis*, and *P. jayakari*, inhabiting seasonally dry environments

in the Arabian Peninsula (Oman and Saudi Arabia), shared a similar globule surface with a sparsely nubby texture and dimples. In contrast, among exemplar species of *Phrynichus* inhabiting tropical rainforest areas with more constant humidity and regular rainfall, such as *P. exophthalmus* from West and central Africa, *P. orientalis* from Southeast Asia, and *P. ceylonicus* from Sri Lanka, the nubby texture of the globule surface was more regular and denser without space for many dimples.

Phrynidae possessed the greatest diversity in cerotegument morphology, including globule surfaces with wrinkled structures (with or without dimples), particle networks, and honeycombed textures. The colloidal particles were either connected or disconnected and consisted mostly of large or small crystals. Their unique, complex shape enabled unambiguous identification among Euamblypygi.

Unlike Phrynichidae, in which the cerotegument structure was generally similar among congeners and, with few exceptions (e.g., *X. machadoi* or *Damon* species with wrinkled globule surface), did not permit species delimitation, interspecific variation in cerotegument structure was pronounced among Phrynidae, and especially *Phrynus*, in which these characters offer the greatest utility for systematics. Even congeneric species exhibited high diversity in cerotegument structures, as observed in the exemplar species of *Phrynus*, *P. damonidaensis*, *P. decoratus*, and *P. kennidae* (Wolff et al., 2017) or *P. longipes* and *Phrynus* sp. Monseñor Nouel. Furthermore, among the exemplar species of *Phrynus*, a clear distinction was evident in the cerotegument structure among species from the Caribbean versus Central America. The latter, represented by *P. maesi* and *P. whitei*, exhibited a wrinkled globule surface with dimples, and colloidal particles comprising large wrinkled to lamelliform crystals, whereas in *P. parvulus*, connecting fibers between globules were present, characters not observed in other Phrynidae.

In *Paraphrynus*, the cerotegument is presently known from four of the seven species assigned to the *aztecus* group (Seiter et al., 2020). In each case, the globule surface exhibited a similar pattern but varied from a wrinkled surface to a particulate network with dimples and pores. Hence, cerotegument structure is consistent across this species complex and could potentially be used for its delimitation. However, the cerotegument structure of *Paraphrynus laevifrons*, the type species of the genus, shares more similarities with *Heterophrynus* than with other congeners.

All three exemplar species of *Heterophrynus* exhibited a distinct cerotegument with a rather smooth, slightly wrinkled globular surface (cf. Wolff et al., 2017). The cerotegument of the monotypic genus *Acanthophrynus* was more similar to that of *Heterophrynus* than to *Paraphrynus* and *Phrynus*, to which it is considered more closely related according to phylogenetic analyses (Schramm et al., 2021; Weygoldt, 1996), perhaps another example of symplesiomorphy.

4.2 | Intraspecific variation

Ultrastructural analysis of the cerotegument of different populations from 13 species in three families, Phrynidae (9), Phrynichidae (3), and Charontidae (1), revealed no major differences. Minor differences observed among some conspecific populations appear to be due to environmental effects on the assembly and hardening of the cerotegument after molting (Filippov et al., 2017; Wolff et al., 2016). Low-intraspecific variation supports the utility of cerotegument characters for the systematics of Amblypygi.

4.3 | Contaminants

When interpreting scanning electron micrographs of the cerotegument, it is important to be aware that contaminants such as microorganisms and environmental inclusions are common. In the present study, many exuviae were obtained from captive individuals and some contaminants may result from the substrata used in terraria. However, contaminants may also occur on individuals preserved in the field, for example, particles originating from other soil organisms such as fungi and collembolans (Gibbons et al., 2019; Wolff et al., 2017). A previous study demonstrated that fungal hyphae grow inside the cerotegumental layer and appear to be tolerated (Gibbons et al., 2019). This phenomenon is ubiquitous, suggesting that a commensal relationship between whip spiders and fungi may exist. According to Gibbons et al. (2019), the relationship is not species-specific and the composition of the cerotegumental mycobiome depends on the environment, that is, the occurrence of fungi in the substrate of the microhabitat. Fungal cells may be distinguished from cerotegumental structures by their simple cellular shapes, often forming filamentous chains (hyphae). Recently, it was shown that hyphae of some mildew species might generate surface superhydrophobicity (Gorb & Gorb, 2021). Therefore, one can hypothesize that fungal hyphae on the whip spider cuticle might contribute to the superhydrophobicity of the cuticle surface. The cells of hyphae are often collapsed and usually have a smooth surface, although some cells exhibit spiked surfaces. Fungal spores may be globular in shape, but differ in size from cerotegument globules, are usually smooth, and often clustered. Particles, including nanoparticles, that appear to be attached to the upper surface of the cerotegument, are irregularly dispersed, only occur locally, and are not embedded in the cerotegument matrix, are probably environmental and do not result from the whip spider secretions.

4.4 | Conclusions

Previous studies revealed that the globules self-assemble after the secretion of the initially fluid cerotegument in the initial days after molting (Wolff et al., 2016, 2017). The form, density, and surface texture of the cerotegument was proven to be highly complex and lineage-specific, with a diversity of shape and arrangement of colloidal nanoparticles. The present study provides the first comparative assessment of cerotegument structure across all genera of Euamblypygi, confirming the hypothesis of Wolff et al. (2017) that the morphology of the colloidal particles and their globules on the surface

differ considerably among taxa and offer informative characters for whip spider systematics, especially within Neoamblypygi.

Weygoldt (1996) hypothesized that Charinidae and Charontidae are the early branching lineages of Euamblypygi and that Phrynidae and Phrynichidae are later branching in the phylogeny. Wolff et al. (2017) suggested an evolutionary trend in the development of cerotegument structures toward an increased complexity, based on the phylogenetic hypothesis of Weygoldt (1996). The present study confirms these findings with a comprehensive sampling of the major clades of Euamblypygi. Unfortunately, no data could be obtained from the sister-group, Paracharontidae, represented by a single extant species of which few specimens exist in museum collections. However, comparison with the cerotegument of Thelyphonida, representing the sister group of Amblypygi, supports this trend. Most species of Thelyphonida exhibit irregular or amorphous cerotegument structures, occasionally globules, but no colloidal nanoparticles (Seiter et al., 2021; Wolff et al., 2017).

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AUTHOR CONTRIBUTIONS

Michael Seiter: Conceptualization (lead); formal analysis (lead); investigation (lead); project administration (lead); visualization (lead); writing – original draft (lead); writing – review and editing (lead). **Thomas Schwaha:** Formal analysis (supporting); methodology (lead); writing – review and editing (supporting). **Lorenzo Prendini:** Formal analysis (supporting); resources (lead); writing – review and editing (supporting). **Stanislav N. Gorb:** Formal analysis (supporting); resources (supporting); writing – review and editing (supporting). **Jonas Wolff:** Conceptualization (supporting); formal analysis (equal); investigation (equal); methodology (supporting); writing – review and editing (equal).

PEER REVIEW

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DATA AVAILABILITY STATEMENT

Data available on request from the authors; The data that support the findings of this study are available from the corresponding author upon reasonable request

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