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Ultrastructural comparison of the eyespot and ocelli of scorpions, and implications for the systematics of Chaerilidae Pocock, 1893☆

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ABSTRACT

An eyespot, a smooth cuticular structure situated posteroventral or ventral to the lateral ocelli, has been documented in at least 60 species in 46 genera and six families of scorpions. The eyespot was confirmed to be absent in 89 genera, 115 species and seventeen families. In the present contribution, the ultrastructure of the eyespot, median and lateral ocelli of the Vietnamese chaerilid scorpion, *Chaerilus julietteae* Lourenço, 2011, is investigated using transmission electron microscopy. As in the buthid scorpion, *Parabuthus transvaalicus* Purcell, 1899, the eyespot of *C. julietteae* differs from the median and lateral ocelli in lacking a dioptric apparatus and pigment granules in the retinula and pigment cells, implying that its function is nonvisual. The presence of an eyespot is documented across Chaerilidae Pocock, 1893, confirming that it is diagnostic and probably synapomorphic for the family. Based on ultrastructure and position, the eyespot of scorpions appears to be a highly modified lateral ocellus that lost its lens and visual function during disintegration of the compound lateral eye.

1. Introduction

An eyespot, also known as an accessory eye, is a smooth cuticular structure situated posteroventral or ventral to the lateral ocelli in many scorpion taxa (Loria and Prendini, 2014). Eyespots have been observed in 60 species, 46 genera and six families of scorpions (Table 1). The eyespot varies in color, size, shape, and position among these taxa. The eyespot of Buthidae C.L. Koch, 1837 is large, white to yellow in color, elongated, and located ventral and posteroventral to the lateral ocelli. It is bright yellow, round to oblong, similar in size and posteroventral to the posterolateral major ocellus (PLMa) in Chaerilidae Pocock, 1893 and some Iuridae Thorell, 1876, and reduced, round and ventral to the posterodorsal minor ocellus (PDMi) in Troglotayosicidae Lourenço, 2008, Vaejovidae Thorell, 1876 and some Iuridae. The eyespot is uncommon in Chactidae Pocock, 1893, and was only observed in some *Nullibrotheas alleni* (Wood, 1863). The eyespot has been confirmed to be absent in 89 genera, 115 species and seventeen families (Table 2).

Across Arachnida, eyespots have been observed in Acari (André and Fain, 2000), Amblypygi (Do Monte et al., 2015; Vasconcelos et al., 2016), Opiliones (Schwendinger and Giribet, 2005), Pseudoscorpiones (Muchmore, 1984; Harvey, 1991), Schizomida (Sissom, 1980; Miether and Dunlop, 2016), Solifugae (Bird et al., 2015, this issue), and possibly Ricinulei (Alberti et al., 2008; Miether and Dunlop, 2016). However, the homology of these eyespots has not yet been assessed. Given its similar position and ultrastructure, the scorpion eyespot may be homologous to the rudimentary 'accessory eye' in the horseshoe crab, *Limulus polyphemus* L., 1758 or one of the lateral ocelli of arachnids (Spreitzer and Melzer, 2003; Lehmann and Melzer 2013).

Scorpion lateral ocelli have biconvex lenses and are arranged in pairs anterolaterally on either side of the carapace (Yang et al., 2013; Loria and Prendini, 2014). After examination of 519 individuals representing 160 genera (78%), 196 species (9%) and nineteen families, Loria and Prendini (2014) proposed a six-ocellus model for the scorpion lateral ocelli, identifying six common ocelli that formed nine general patterns across scorpions. Four additional accessory ocelli were rarely observed and therefore excluded from the general patterns identified. The maximum number of lateral ocelli recorded in a single species was eight. Experimental studies on the buthid, *Androctonus australis* (L., 1758), indicate that the lateral ocelli can perceive differences in brightness at very low intensities, serving as light detectors

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Table 1
Recent scorpion families and genera in which eyespot is present (Loria and Prendini, 2014).

Family	Genus	Exemplar Species
Buthidae C.L. Koch, 1837	*Afroisometrus* Kovařík, 1997	1
	Alayotityus Armas, 1973	1
	Babycurus Karsch, 1886	1
	Butheolus Simon, 1882	1
	Buthoscorpio Werner, 1936	2
	Buthus Leach, 1815	1
	Centruroides Marx, 1890	1
	Cicileus Vachon, 1948	1
	Grosphus Simon, 1880	1
	Isometroides Keyserling, 1885	1
	Isometrus Ehrenberg, 1828	2
	Karasbergia Hewitt, 1913	1
	Lychas C.L. Koch, 1845	2
	Microbuthus Kraepelin, 1898	1
	Neobuthus Hirst, 1911	1
	Neogrosphus Lourenço, 1995	1
	Odonturus Karsch, 1879	1
	Orthochirus Karsch, 1891	1
	Parabuthus Pocock, 1890	1
	Physoctonus Mello-Leitão, 1934	1
	Pseudolychas Kraepelin, 1911	1
	Rhopalurus Thorell, 1876	1
	Sassanidotus Farzanpay, 1987	1
	Thaicharmus Kovařík, 1995	1
	Tityopsis Armas, 1974	1
	Uroplectes Peters, 1861	1
	Zabius Thorell, 1893	1
Chactidae Pocock, 1893	*Nullibrotheas* Williams, 1974	1
Chaerilidae Pocock, 1893	*Chaerilus* Simon, 1877	10
Iuridae Thorell, 1876	*Caraboctonus* Pocock, 1893	1
	Hadruroides Pocock, 1893	1
	Hadrurus Thorell, 1876	1
	Hoffmannihadrurus Fet & Soleglad, 2004	2
Troglotayosicidae Lourenço, 1998	*Troglotayosicus* Lourenço, 1981	1
Vaejovidae Thorell, 1876	*Franckeus* Soleglad & Fet, 2005	1
	Gertschius Graham & Soleglad, 2007	1
	Kochius Soleglad & Fet, 2008	1
	Maaykuyak González-Santillán & Prendini, 2013	1
	Mesomexovis González-Santillán & Prendini, 2013	1
	Paruroctonus Werner, 1934	1
	Pseudouroctonus Stahnke, 1974	1
	Serradigitus Stahnke, 1974	1
	Stahnkeus Soleglad & Fet, 2006	1
	Syntropis Kraepelin, 1900	1
	Thorellius Soleglad & Fet, 2008	1
	Vaejovis C.L. Koch, 1836	2
	Total	60

(Machan, 1968a,b; Fleissner, 1977d; Schliwa and Fleissner, 1980; Hjelle, 1990; Warburg and Polis, 1990; Root, 1990; Lehmann and Melzer, 2013). Therefore, the lateral ocelli probably detect *Zeitgeber* stimuli and synchronize the circadian rhythm (Fleissner, 1975; Fleissner, 1977a,b,c,d; Schliwa and Fleissner, 1980; Hjelle, 1990; Warburg and Polis, 1990; Lehmann and Melzer, 2013). For acuity and spatial discrimination, scorpions rely on the median ocelli (Schliwa and Fleissner, 1980; Hjelle, 1990; Lehmann and Melzer, 2013), although these ocelli can also detect *Zeitgeber* stimuli (Fleissner, 1977a,b,c). All except 26 troglomorphic scorpion species possess a single pair of median ocelli with biconvex lenses, situated dorsomedially on the carapace (Fig. 1A; Loria and Prendini, 2014).

Spreitzer and Melzer (2003) compared the ultrastructure of the eyespot (referred to as an accessory eye) with the lateral ocelli of another buthid, *Parabuthus transvaalicus* Purcell, 1899, using transmission electron microscopy (TEM) and discovered that the eyespot possesses pigment cells, retinula cells with rhabdomeres, and arhabdomeric cells like the lateral ocelli. Unlike the lateral ocelli, the eyespot of *P. transvaalicus* lacks a dioptric apparatus and pigment granules in the retinula and pigment cells, implying that its function is nonvisual (Spreitzer and Melzer, 2003).

The scorpion family Chaerilidae includes 42 species in a single genus, *Chaerilus* Simon, 1877, distributed across South and Southeast Asia and one putative fossil species, *Electrochaerilus buckleyi* Santiago-Blay et al., 2004, dated to the lower Cretaceous (Kovařík, 2000; Santiago-Blay et al., 2004; Lourenço, 2011; Kovařík and Ojanguren Affilastro, 2013; Kovařík et al., 2014,2015; Lourenço and Pham, 2014; Yin et al., 2015). Chaerilidae are unquestionably monophyletic (Stockwell, 1989; Coddington et al., 2004) and characters commonly used to diagnose the family include the Type B trichobothrial pattern, fusiform hemispermatophore, circular stigmata, two pairs of lateral ocelli and unique cheliceral dentition (Vachon, 1974; Sissom, 1990; Fet, 2000). The presence of an eyespot has occasionally been suggested as a diagnostic character for the family. Kraepelin (1894: 7) was the first to identify the eyespot as diagnostic for Chaerilini Pocock, 1893, referring to it as a 'bright yellow spot' behind the posterior ocellus, and used it again as a diagnostic character when elevating Chaerilini to the rank of family (Kraepelin, 1899). Pocock (1893, 1900) referred to two pairs

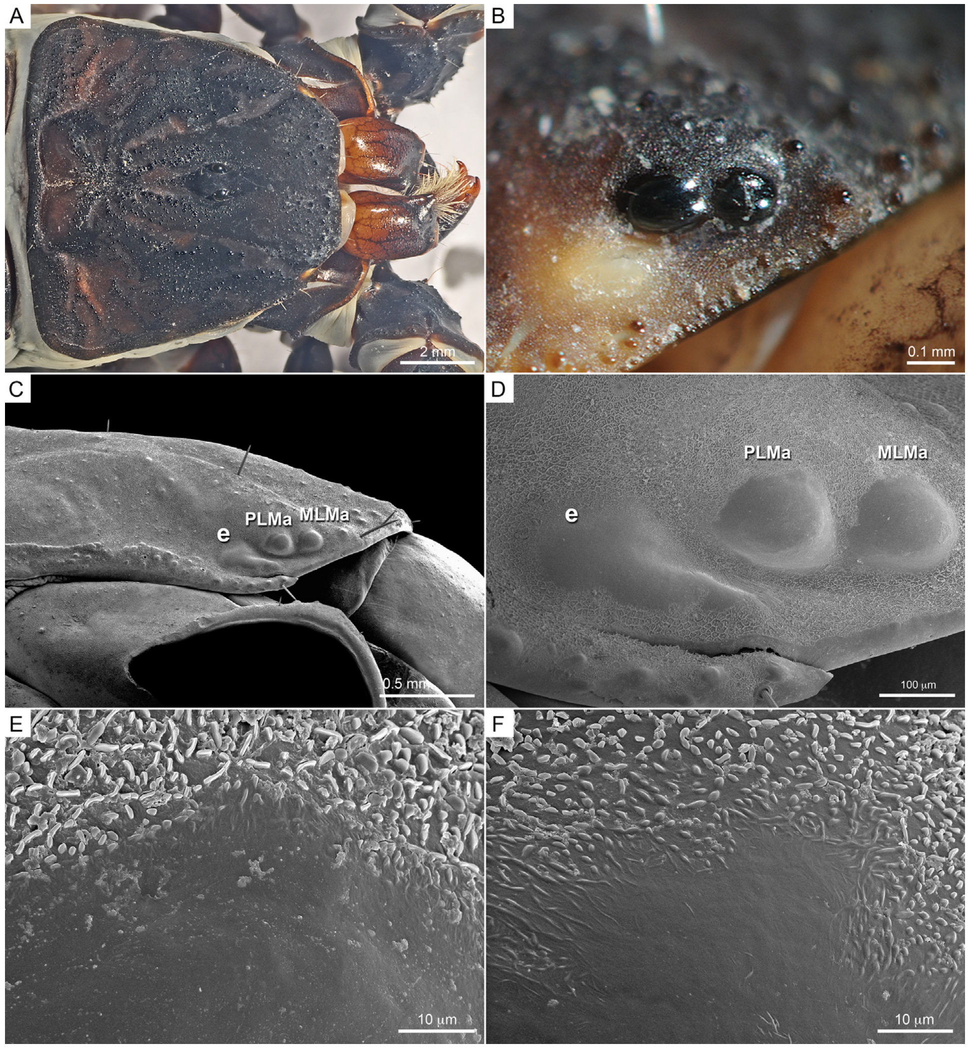

Fig. 1. *Chaerilus julietteae* Lourenço, 2011, gross morphology and positions of ocelli and eyespot. A, B. Carapace, dorsal and lateral views depicting median ocelli (A), lateral ocelli, and eyespot (B). C, D. Scanning electron micrographs of eyespot (e), posterolateral major ocellus (PLMa) and posterolateral major ocellus (MLMa). E, F. Scanning electron micrographs comparing surfaces of carapace, PLMa (E) and eyespot (F).

of lateral ocelli in his diagnosis of Chaerilidae, but did not mention the eyespot. Tikader and Bastawade (1983) referred to an 'amber-coloured spot' below the two pairs of lateral ocelli in their diagnosis of Chaerilidae and used the character to separate the family from Vaejovidae. The eyespot was not mentioned as a diagnostic character in subsequent works concerning the family (Stockwell, 1989; Sissom, 1990; Kovařík, 2000; Soleglad and Fet, 2003; Kovařík and Ojanguren Affilastro, 2013).

In the present contribution, the ultrastructure of the eyespot, median ocelli and lateral ocelli of the chaerilid, *Chaerilus julietteae* Lourenço, 2011, is investigated using TEM. Results are compared with a previous study of the eyespot and lateral ocelli of a buthid, *P. transvaalicus*, by Spreitzer and Melzer (2003). The presence of an eyespot is documented across Chaerilidae Pocock, 1893, confirming that it is diagnostic and probably synapomorphic for the family.

2. Materials and methods

2.1. Material examined

The presence of an eyespot was verified in 733 individuals representing 39 (93%) of the described species of *Chaerilus* Simon, 1877. Two juvenile specimens of *Chaerilus julietteae* Lourenço, 2011, from the type locality, Ta Kou Mountain, Vietnam, were used for SEM and TEM.

2.2. Scanning and transmission electron microscopy

One specimen was sectioned with a razor for histology. Primary fixation of the carapace was performed by immersion in 3.5% glutaraldehyde buffered with 0.1 M phosphate buffer (pH = 7.4) for 24 h. Secondary fixation was performed by immersion in osmium tetroxide for 2–4 h. Specimens were washed with 0.1 M phosphate buffer between fixations. Tissue was dried using HMDS solution and sputtered with gold. Scanning electron microscopy (SEM) was conducted using a Hitachi S4700 FE SEM at the Microscopy and Imaging Facility of the American Museum of Natural History. The second specimen was fixed by immersion in 0.1 M sodium cacodylate buffer (pH 7.2) containing 2.5% glutaraldehyde and 2% paraformaldehyde and post-fixed by immersion in 1% osmium tetroxide for 1.5 h at ambient temperature, then embedded in EMbed 812 (Electron Microscopy Sciences, Hatfield, PA). 500 nm semithin sections were cut, stained with 1% toluidine blue, and imaged with a Zeiss Axio Scope A1 microscope. Ultrathin sections (70 nm) were cut, mounted on copper grids and stained with uranyl acetate and lead citrate using standard protocols. Stained grids were examined under a Philips CM-12 transmission electron microscope (FEI, Eindhoven, The Netherlands) and photographed with a Gatan (4 k × 2.7 k) digital camera (Gatan, Inc., Pleasanton, CA).

Table 2
Recent scorpion families and genera in which eyespot is absent (Loria and Prendini, 2014).

Family	Genus	Exemplar Species
Akravidae Levy, 2007	*Akrav* Levy, 2007	1
Bothriuridae Simon, 1880	*Bothriurus* Peters, 1861	2
	Brachistosternus Pocock, 1893	1
	Brandbergia Prendini, 2003	1
	Centromachetes Lønnberg, 1897	1
	Cercophonius Peters, 1861	1
	Lisposoma Lawrence, 1928	2
	Orobothriurus Maury, 1975	1
	Pachakutej Ochoa, 2004	1
	Phoniocercus Pocock, 1893	1
	Rumikiru Ojanguren-Affilastro et al., 2012	1
	Thestylus Simon, 1880	1
	Timogenes Simon, 1880	1
	Urophonius Pocock, 1893	1
	Vachonia Abalos, 1954	1
Buthidae C.L. Koch, 1837	*Akentrobuthus* Lamoral, 1976	1
	Ananteris Thorell, 1891	1
	Butheoloides Hirst, 1925	2
	Mesotityus González-Sponga, 2004	1
	Microananteris Lourenço, 2003	1
	Microcharmus Lourenço, 1995	1
	Microtityus Kjellesvig-Waering, 1966	1
	Pseudouroplectes Lourenço, 1995	1
	Tityobuthus Pocock, 1893	1
Chactidae Pocock, 1893	*Broteochactas* Pocock, 1893	1
	Brotheas C.L. Koch, 1837	2
	Chactas Gervais, 1844	1
	Chactopsis Kraepelin, 1912	1
	Chactopsoides Ochoa et al., 2013	1
	Hadrurochactas Pocock, 1893	2
	Megachactops Ochoa et al., 2013	1
	Neochactas Soleglad and Fet, 2003	1
	Taurepania González-Sponga, 2004	2
	Teuthraustes Simon, 1878	1
	Vachoniochactas González-Sponga, 2004	2
Diplocentridae Karsch, 1880	*Bioculus* Stahnke, 1968	2
	Cazierius Francke, 1978	1
	Didymocentrus Kraepelin, 1905	1
	Diplocentrus Peters, 1861	1
	Heteronebo Pocock, 1899	2
	Kolotl Santibañez-Lopez et al., 2014	1
	Nebo Simon, 1878	1
	Oiclus Simon, 1880	1
	Tarsoporosus Francke, 1978	2
Euscorpiidae Laurie, 1896	*Euscorpius* Thorell, 1876	2
	Megacormus Karsch, 1881	1
	Plesiochactas Pocock, 1900	2
	Troglocormus Francke, 1981	2
Hemiscorpiidae Pocock, 1893	*Hemiscorpius* Peters, 1861	1
Heteroscorpionidae Kraepelin, 1905	*Heteroscorpion* Birula, 1903	3
Hormuridae Laurie, 1896	*Cheloctonus* Pocock, 1892	1
	Chiromachetes Pocock, 1899	1
	Chiromachus Pocock, 1893	1
	Hadogenes Kraepelin, 1894	1
	Hormiops Fage, 1933	1
	Hormurus Thorell, 1876	1
	Iomachus Pocock, 1893	2
	Liocheles Sundevall, 1833	1
	Opisthacanthus Peters, 1861	4
	Palaeocheloctonus Lourenço, 1996	1
Iuridae Thorell, 1876	*Anuroctonus* Pocock, 1893	1
	Calchas Birula, 1899	2
	Iurus Thorell, 1876	1
	Neocalchas Yagmur et al., 2013	1
	Protoiurus Soleglad et al., 2012	1
Pseudochactidae Gromov, 1998	*Pseudochactas* Gromov, 1998	1
	Troglokhammouanus Lourenço, 2007	1
	Vietbocap Lourenço and Pham, 2010	1
Scorpionidae Latreille, 1802	*Heterometrus* Ehrenberg, 1828	1
	Opistophthalmus C.L. Koch, 1837	2
	Pandinus Thorell, 1876	1
	Scorpio Linnaeus, 1758	1

Table 2 (*Continued*)

Family	Genus	Exemplar Species
Scorpiopidae Kraepelin, 1905	*Alloscorpiops* Vachon, 1980	1
	Euscorpiops Vachon, 1980	2
	Parascorpiops Banks, 1928	1
	Scorpiops Peters, 1861	1
Superstitionidae Stahnke, 1940	*Superstitionia* Stahnke, 1940	1
Typhlochactidae Mitchell, 1971	*Alacran* Francke, 1982	2
	Sotanochactas Francke, 1986	1
	Stygochactas Vignoli and Prendini, 2009	1
	Typhlochactas Mitchell, 1971	2
Urodacidae Pocock, 1893	*Aops* Volschenk and Prendini, 2008	1
	Urodacus Peters, 1861	2
Vaejovidae Thorell, 1876	*Kovarikia* Soleglad et al., 2014	1
	Pseudouroctonus Stahnke, 1974	1
	Uroctonites Williams and Savary, 1991	1
	Uroctonus Thorell, 1876	2
	Vaejovis C.L. Koch, 1836	1
	Wernerius Soleglad and Fet, 2008	1
	Total	115

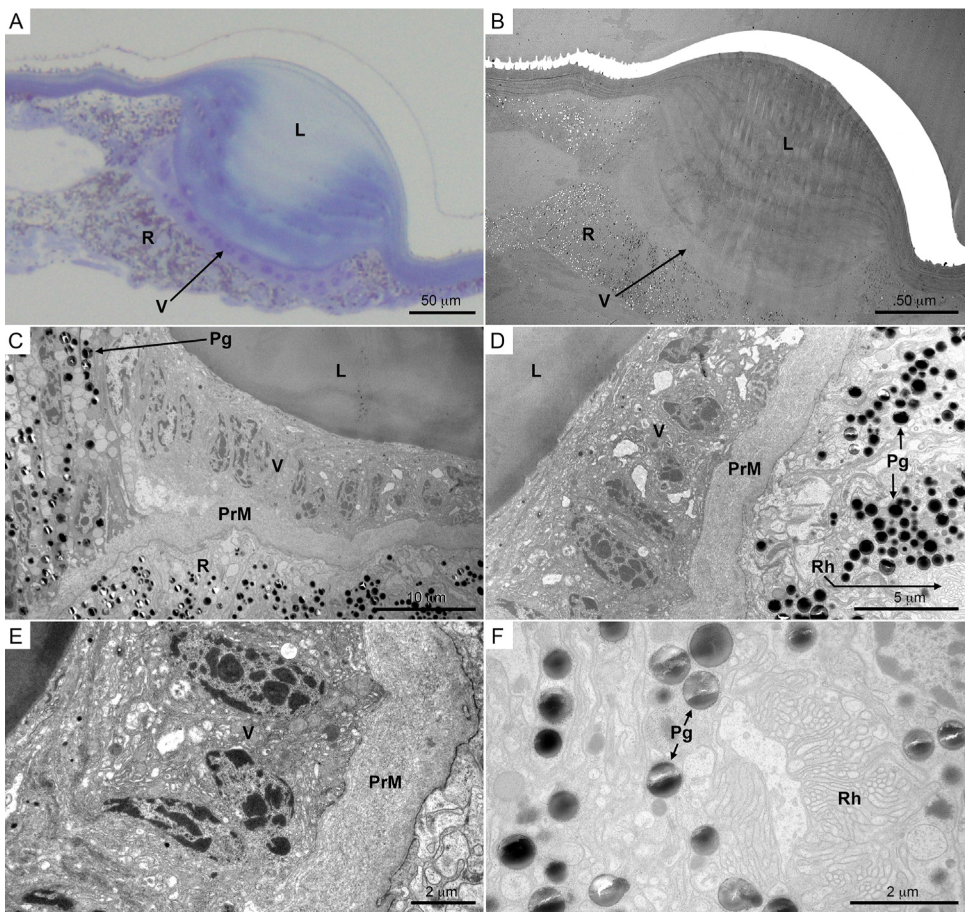

Fig. 2. *Chaerilus julietteae* Lourenço, 2011, ultrastructure of median ocellus. Light microscopy section (A) and transmission electron micrographs (B–F) depicting lens (L), vitreous body (V), preretinal membrane (PrM), and retina (R), which includes retinula and pigment cells with pigment granules (Pg), rhabdomeres (Rh), and arhabdomeric cells.

3. Results

3.1. Gross morphology of eyespot

An eyespot was observed in all chaerilid specimens examined, representing 39 described species. The eyespot is situated posteroventral or ventral to the PLMa (Fig. 1A–D). It is bright yellow and elongated, and as large as or larger than the PLMa. Unlike the median and lateral ocelli, the eyespot lacks a cuticular lens. However, the smooth surfaces of the eyespot and lateral ocelli are almost indistinguishable with SEM (Fig. 1E, F).

3.2. Ultrastructural comparison of eyespot and ocelli

The median ocellus, situated dorsomedially on the carapace, comprises several layers including a lens attached to the cuticle, a vitreous body, preretinal membrane, and retina (Table 3; Fig. 2A, B). The vitreous body is situated between the lens and the preretinal membrane (Fig. 2C) and contains elongated cells (Fig. 2D, E). The retina is separated from the vitreous body by the prereti-

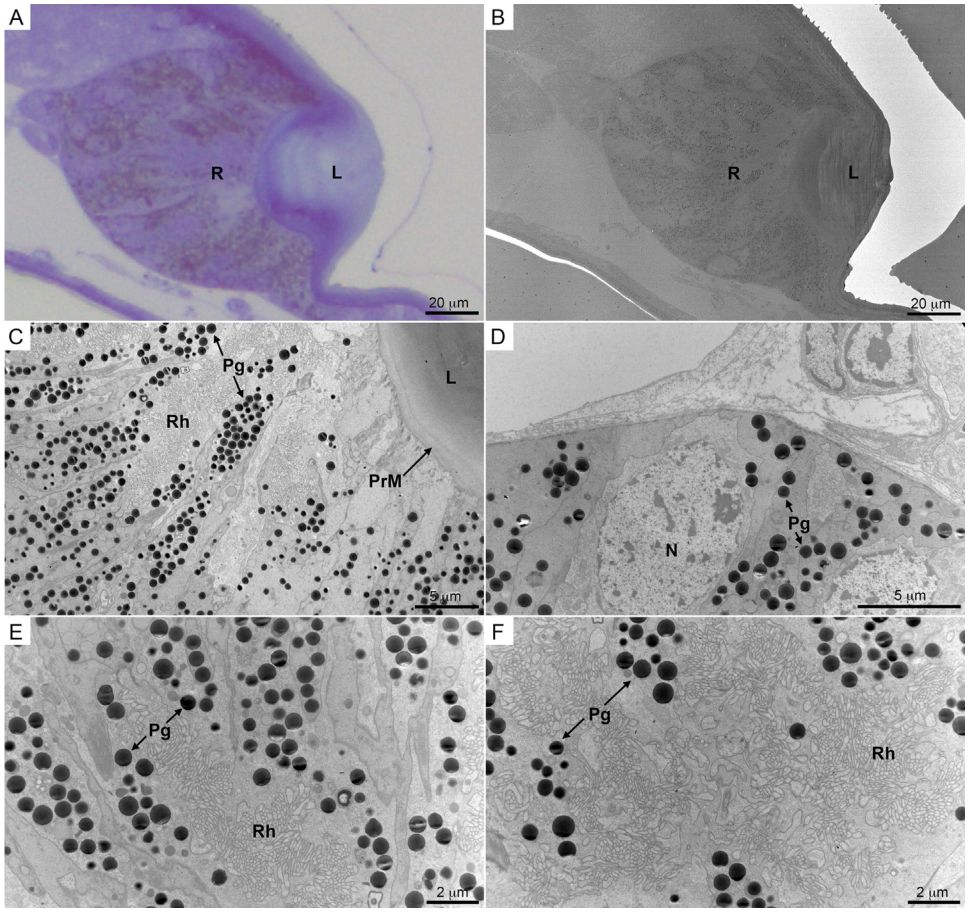

Fig. 3. *Chaerilus julietteae* Lourenço, 2011, ultrastructure of lateral ocellus. Light microscopy section (A) and transmission electron micrographs (B–F) depicting lens (L) and retina (R), which includes retinula and pigment cells with pigment granules (Pg), rhabdomeres (Rh), cell nuclei (N), and arhabdomeric cells.

nal membrane and contains retinula cells with rhabdomeres that form fingerlike projections known as microvilli, and pigment cells with pigment granules (Fig. 2F). The lateral ocellus comprises a lens, preretinal membrane and retina but lacks a vitreous body (Table 3; Fig. 3A, B). The retinula cells form a network of rhabdomeres interspersed with pigment granules (Fig. 3C, E, F). Large nuclei are present in the retinula cells (Fig. 3D) and rhabdomeres form microvilli as in the median ocelli (Fig. 3C, E, F). The eyespot only includes a retina and lacks a vitreous body like the lateral ocelli, but also lacks a lens and preretinal membrane (Table 3; Fig. 4A, B). Retinula cells with rhabdomeres are present but lack pigment granules (Fig. 4D, E). Arhabdomeric cells are also present and situated adjacent to the retinula cells (Fig. 4C). The rhabdomeres form a network of microvilli (Fig. 4E, F).

Table 3
Ultrastructural comparison of eyespot and ocelli of *Chaerilus julietteae* Lourenço, 2011.

Structure	Median Ocellus	Lateral Ocellus	Eyespot
Lens	Present	Present	Absent
Vitreous Body	Present	Absent	Absent
Pigment cells	Present	Present	Present
Arhabdomeric cells	Present	Present	Present
Retinula cells	Present	Present	Present
Rhabdomeres	Present	Present	Present
Pigment granules	Present	Present	Absent

4. Discussion

4.1. *Eyespot diagnostic and synapomorphic for Chaerilidae*

The presence of an eyespot was not conclusively established as a diagnostic character for Chaerilidae until now. Kraepelin (1894, 1899) and Tikader and Bastawade (1983) referred to an amber or yellow spot near the lateral ocelli in their diagnoses but many other authors ignored the structure (Pocock, 1893, 1900; Stockwell, 1989; Sissom, 1990; Kovařík, 2000; Soleglad and Fet, 2003; Kovařík and Ojanguren Affilastro, 2013). Loria and Prendini (2014) defined the lateral ocellus pattern in Chaerilidae as Type 2A, comprising a mediolateral major ocellus (MLMa) and a posterolateral major ocellus (PLMa), but also observed several aberrant conditions including a single ocellus, Type 1 (PLMa), or three ocelli, Type 2A with either an anterolateral major ocellus (ALMa) or a posterolateral minor ocellus (PLMi). Despite these differences, an eyespot was observed in all individuals examined.

In the present study, an eyespot was observed in all 733 individuals examined, representing 39 (93%) species of Chaerilidae, confirming that it is ubiquitous across the family. Comparison of the chaerilid eyespot with the eyespots of other scorpion taxa revealed differences in shape, size and coloration. The eyespot of Buthidae is larger, paler and more elongate, extending posteroventral and ventral to the lateral ocelli. The eyespots of *Troglotayosicus* Lourenço, 1981, twelve genera of Vaejovidae, and at least two species of Iuridae, *Hoffmannihadrurus gertschi* (Soleglad, 1976) and *Hadrurus arizonensis* Ewing, 1928 are smaller, more rounded, and situated ventral to the PDMi. The eyespots of *Caraboctonus keyserlingii* Pocock, 1893 and *Hadruroides charcasus* (Karsch, 1879) are the most similar in shape, size and color to the eyespot of Chaerilidae, but differ in being situated ventral to the PDMi, which is absent in

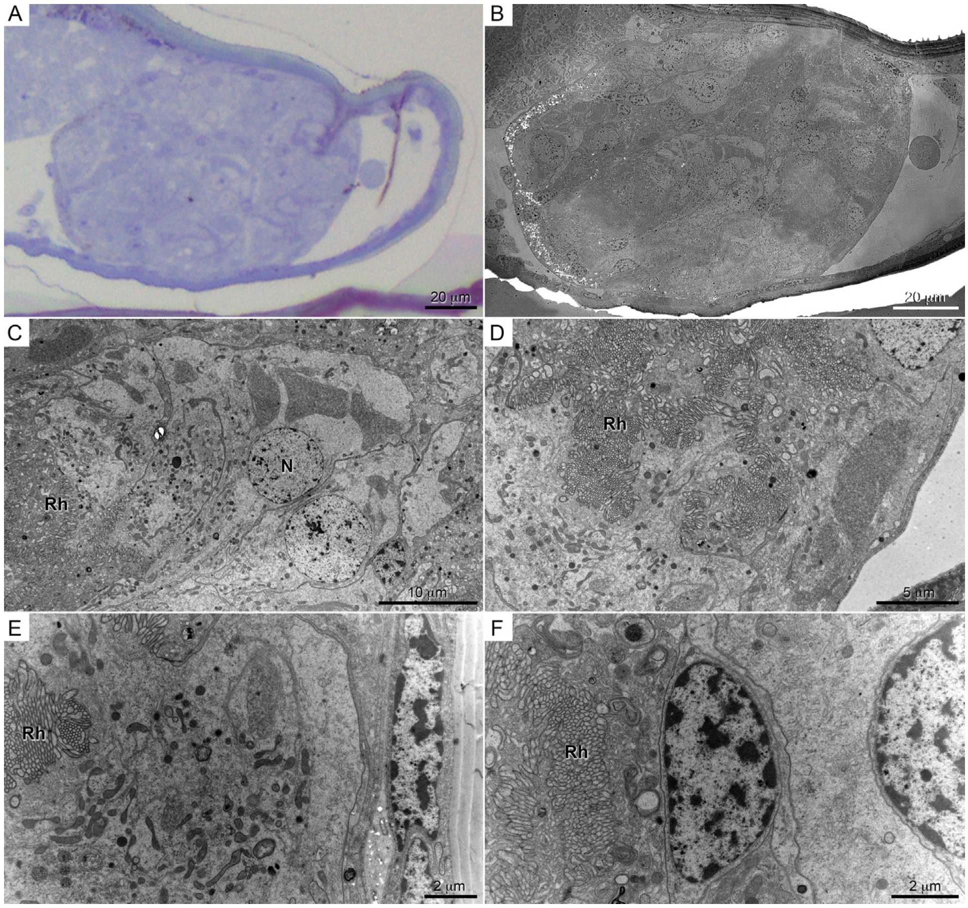

Fig. 4. *Chaerilus julietteae* Lourenço, 2011, ultrastructure of eyespot. Light microscopy section (A) and transmission electron micrographs (B–F) depicting retinula and pigment cells with rhabdomeres (Rh), cell nuclei (N), and arhabdomeric cells.

Chaerilidae. The unique shape and position of the chaerilid eyespot suggest that it is probably synapomorphic for the family.

4.2. Function of eyespot in scorpions

The ultrastructure of the eyespot, median and lateral ocelli of *C. julietteae* agree with previous investigations of these structures in other taxa (Graber, 1879; Lankester and Bourne, 1883; Parker, 1887; Police, 1907; Scheuring, 1913; Werner, 1934; Machan, 1966, 1967; Bedini, 1967; Fleissner, 1972, 1974, 1985; Belmonte and Stensaas, 1975; Fleissner and Schliwa, 1977; Schliwa and Fleissner, 1979, 1980; Locket, 1986; Hjelle, 1990; Root, 1990; Spreitzer and Melzer, 2003; Lehmann and Melzer, 2013). The median ocelli are divided into several layers including a lens, vitreous body, preretinal membrane, retina, postretinal membrane, and a layer of pigment cells surrounding the retina (Graber, 1879; Lankester and Bourne, 1883; Parker, 1887; Police, 1907; Scheuring, 1913; Werner, 1934; Machan, 1966, 1967; Bedini, 1967; Fleissner, 1972, 1974; Fleissner and Siegler, 1978; Schliwa and Fleissner, 1979, 1980; Locket, 1986; Hjelle, 1990; Root, 1990; Lehmann and Melzer, 2013; Warburg, 2013). In contrast, the lateral ocelli possess a lens, preretinal membrane, retina, and postretinal membrane, but lack a vitreous body (Graber, 1879; Lankester and Bourne, 1883; Parker, 1887; Police, 1907; Scheuring, 1913; Werner, 1934; Machan, 1966, 1967; Bedini, 1967; Schliwa and Fleissner, 1980; Hjelle, 1990; Root, 1990; Spreitzer and Melzer, 2003; Lehmann and Melzer, 2013; Warburg, 2013). Additionally, the retinula cells of the lateral ocelli do not form discrete units as in the median ocelli, and their rhabdomeres form a contiguous rhabdom implying continuous signaling among cells (Schliwa and Fleissner, 1980; Hjelle, 1990; Root, 1990; Lehmann and Melzer, 2013). The optic nerve assembles inside the retina of the median ocelli, whereas it assembles outside the retina of the lateral ocelli (Schliwa and Fleissner, 1980). The median ocelli also possess three to six neurosecretory fibers per retinula cell whereas the lateral ocelli possess approximately one per retinula cell (Schliwa and Fleissner, 1980).

As in *P. transvaalicus*, the eyespot of *C. julietteae* lacks a lens and contains retinula cells with rhabdomeres and pigment cells, but lacks pigment granules. As suggested by Spreitzer and Melzer (2003), the absence of a lens and pigment granules implies that the function of the eyespot is nonvisual. Lenses are essential for concentrating light into ocelli and pigment granules prevent light from scattering among rhabdomeres (Machan, 1968a, 1968b; Mancillas and Brown, 1984; Fleissner, 1986; Spreitzer and Melzer, 2003; Bitsch and Bitsch, 2005). The eyespot may play an ancillary role in processing visual information from the lateral ocelli (Spreitzer and Melzer, 2003).

4.3. Origin of eyespot in scorpions

Early fossil scorpions possessed compound or holochroal lateral eyes comprising many ommatidia sharing a common cornea (Kjellesvig-Waering, 1986; Sissom, 1990; Miether and Dunlop, 2016). For example, *Proscorpius osborni* (Whitfield, 1885) from the Silurian possessed compound eyes with up to 1000 ommatidia (Dunlop et al., 2008). However, later scorpion fossils show the compound eye fragmenting into smaller parts with fewer lenses (Kjellesvig-Waering, 1986; Sissom, 1990; Bitsch and Bitsch, 2005; Paulus, 1979, 2000; Miether and Dunlop, 2016). Miether and Dunlop (2016) confirmed that the Triassic scorpions, *Mesophonus opisthophthalmus* Wills, 1947 and *Mesophonus peronatus* Wills, 1910, possessed 28 and 35 lenses, respectively. Other recent fossil scorpions, such as the Carboniferous *Compsoscorpius* Petrunkevitch, 1999 and *Palaeopisthacanthus* Petrunkevitch, 1913, possessed three pairs of lateral ocelli (Stockwell, 1989; Sissom, 1990; Jeram, 1994, 1998), closely resembling living scorpions. Based on ultrastructure and position, the eyespot of scorpions appears to be a highly modified lateral ocellus that lost its lens and visual function during disintegration of the compound lateral eye (Spreitzer and Melzer, 2003).

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