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# A study of common scorpionfly (Mecoptera: Panorpidae) visual systems reveals the expression of a single opsin

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**Abstract** Knowledge of insect color vision and the genes that support color vision has been growing recently. Yet, research on some groups is limited (e.g., Mecoptera). Common scorpionflies (Panorpidae) are highly visual insects with many intriguing behaviors. We hypothesized that the family Panorpidae employs a complex color vision system and predicted that multiple opsin classes are expressed in the lineage. Transcriptomes were generated from the eye tissues for two species of Panorpidae (*Panorpa acuminata* and *P. nebulosa*) and one species of Boreidae (*Boreus coloradensis*). Opsins isolated from the transcriptomes were combined in a phylogenetic analysis with opsin sequences from other insect orders (e.g., those that are sensitive to ultraviolet, blue, and long wavelength light as part of the photopigment). A single long-wavelength opsin sequence was recovered from the panorpid species, while all three opsin classes (ultraviolet, blue, and long-wavelength) were recovered from the boreid. Among insects, this represents a potential case of monochromy due to a loss of opsin gene expression in the blue and ultraviolet portions of the visible light spectrum.

**Keywords** Boreidae · Transcriptome · Phototransduction · Photopigment · Rhodopsin

## Introduction

Entomologists have been interested in insect vision for more than 200 years (de Serres 1815). Despite this long history of insect visual system research, relatively little is known about the visual systems for the majority of insect groups, especially at the molecular level (Porter et al. 2011). A sharper picture of visual systems is beginning to take shape in a few groups (e.g., butterflies, flies, and bees; (Spaethe and Briscoe 2004; Karalius and Buda 2007; Kashiyama et al. 2009; Briscoe et al. 2010). However, even among these insect groups, when one considers the sheer diversity of species (e.g., >20,000 butterfly and >25,000 bee species), only a few representatives of each order have been studied, leading to poor understanding about the overall evolution of their visual systems.

Insects rely on vision for survival and sexual communication (Briscoe et al. 2010; Bybee et al. 2012; Doering et al. 2012). Color vision is one of the main senses used by insects for intra- and interspecific communication and detection of prey and/or food sources. The ancestral insect is hypothesized to have one copy of each of the three known classes of opsins that support color vision: an ultraviolet sensitive (UV), a blue sensitive, and a long wavelength sensitive (LW) gene (Briscoe and Chittka 2001; Porter et al. 2011). Among major insect lineages, the number of opsins can vary at both the ordinal and familial levels. For example, some butterfly species may have up to six opsins that are expressed in the eye (Briscoe 2000), while loss of opsin gene function (resulting in limited color discrimination) has also been investigated in Siphonaptera and their close relatives from Mecoptera (Taylor et al. 2005).

### Mecopteran vision

There is limited data on the color visual systems of common scorpionflies. Mecoptera is a small holometabolous insect order of ~550 species placed within nine families. They are moderately diverse in morphology and display adult behaviors such as hanging from vegetation (Bittacidae) or appearing only in

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wintertime, and living on mosses (Boreidae). Boreidae is a small phylogenetically primitive mecopteran family that also appears to be closely related to fleas (Whiting 2002; Burrows 2011). Little research has been done on boreid ecology or behavior and still less is known concerning their phylogenetics (Whiting et al. 1997; Whiting 2002; Beutel et al. 2008; Friedrich et al. 2013). In regard to vision, boreids have complex eyes and three ocelli. The molecular composition of the opsins expressed in the boreid eye is limited to one study (Taylor et al. 2005)

The family Panorpidae (>375 species) represents both the most diverse and derived lineages within Mecoptera. They are an ancient group of insects (>270 MYO; Byers 1969; Thornhill 1980a, b) with a unique adult morphology. They are characterized by the elongate rostrum and distinct wing banding on sometimes colorful wings (Fig. 1a–d; Byers and Thornhill 1983) and their taxonomy has been studied extensively (Esben-Petersen 1921; Issiki 1933; Snodgrass 1935; Ferris and Rees 1939; Byers 2011; Krzeminski and Soszynska-Maj 2012). Research has focused on their behavior and ecology, particularly in regard to courtship, mating, and feeding behavior (Thornhill 1981; Byers and Thornhill 1983; Engqvist and Sauer 2002; Zhong and Hua 2013). Complex behaviors observed in *Panorpa* include courtship displays, presentation of nuptial gifts, forced copulations, transvestitism, inter- and intraspecific feeding rivalries, and kleptoparasitism of spiders (Thornhill 1980a, b, 1981; Byers and Thornhill 1983; Jennings and Sferra 2002). In addition, panorpids are the most active visual predators in Mecoptera and use their vision to actively hunt for and combat with spiders for prey (Thornhill 1980a, b; Jennings and Sferra 2002). Additionally, male panorpids use their wings to attract the attention of a female by landing on broad leaves and beating their wings up and down (Byers and Thornhill 1983).

It is unclear to what degree panorpid color vision plays in these visually centered behaviors. While the majority of the species have dull colored bodies (one exception is the common US species, *P. nebulosa*), panorpids are well known for color and banding in the wings. With this wing coloration/banding, color vision would seem to have a central role in their behavior in regard to sexual selection and/or ability to hunt as a visual

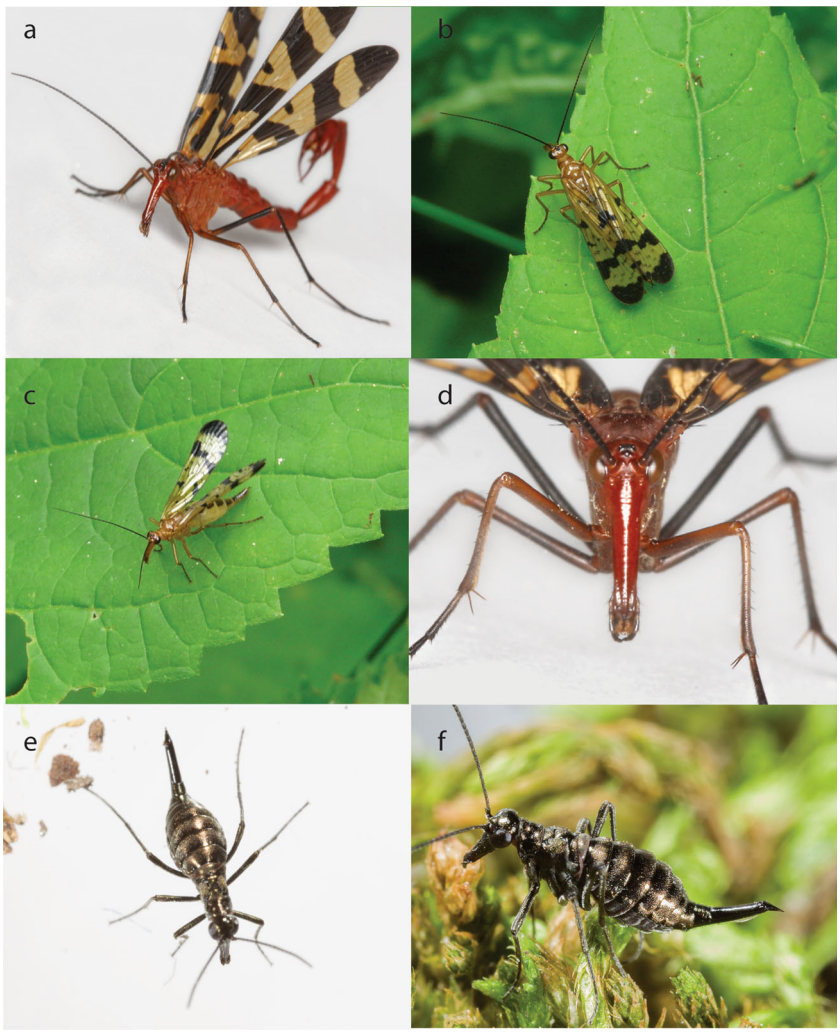


**Fig. 1** **a** *P. nebulosa*, contributed by Gayle Strickland. **b** *P. hungerfordii*, contributed by Ken Sproule. **c** *P. hungerfordii*, contributed by Ken Sproule. **d** *P. nebulosa*, contributed by Gayle Strickland. **e** *B. coloradensis*. **f** *B. coloradensis*

predator. Though there are not any studies on the function of panorpid wing coloration and banding, in other groups (i.e., *Heliconius* and lycaenid butterflies), it has been found that wing coloration is likely connected with color vision (Sison-Mangus et al. 2006; Briscoe et al. 2010). Though panorpids certainly do not have as wide spectrum of coloration as Lepidoptera, their wings do exhibit extensive coloration/banding ranging from transparent to orange and black.

### Mecoptera opsins

There are two studies that represent what is known of mecopteran opsin diversity (Burkhardt and de la Motte 1972; Taylor et al. 2005). These studies employed electroretinograms and complementary DNA (cDNA) to answer research questions regarding panorpid and boreid visual systems, but neither directly investigated the number of opsin classes and copies the group possesses. Burkhardt and de la Motte (1972) used electrophysiology to measure the spectral sensitivity of *P. cognata* and found that its maximum spectral sensitivity peak was at 490–520 nm with a beta peak (signal from cis-formation of the chromophore) at 360 nm. Taylor et al. (2005) used degenerate primers to amplify opsins in six species of panorpids and one species of boreid (*Boreus coloradensis*). They found only the LW opsin using degenerate primers from these taxa.

We present the first molecular data isolated from transcriptomes of mecopteran eye tissue for three taxa (*Panorpa acuminata* (Byers 1993), *P. nebulosa*, and *Boreus coloradensis* (Byers 1955)). Our main objective is to determine if panorpids are capable of polychromatic vision or if there is evidence that they are monochromatic (Burkhardt and de la Motte 1972). Transcriptomes (RNA-seq) of insect eye tissues are useful for identifying opsins (e.g., Bybee et al. 2012). Once generated, the transcriptome can be used to discover the class and copy-number variation of expressed opsins in the eye tissue as well as obtain the entire exonic sequence of each opsin copy.

It is possible that panorpids and boreids may express opsins from the three classes of opsins (UV, Blue, and LW), similar to other insect orders, but at levels that are not detectable physiologically. If this is the case, the biological relevance of the UV and B opsin copies to the Mecoptera visual system is completely unclear. We included Boreidae on the rationale that (1) they represent a primitive mecopteran lineage that is consistently recovered at the base of Mecoptera (Whiting 2002; Misof et al. 2000); (2) boreids appear to be much less visually oriented than panorpids in nature. They are apterous, are non-predatory, and have a uniform, metallic-black coloration. While good at detecting movement, boreids seem to have little "need" to distinguish colors like panorpids; and (3) for comparison purposes with Taylor et al. (2005).

## Methods

### Taxon sampling

Transcriptome sequencing of the visual tissues was conducted on *B. coloradensis* (male) and on two species of *Panorpa*: *P. acuminata* (female) and *P. nebulosa* (female). The boreid was collected in Utah County, Utah and the panorpid specimens were collected from Forsyth County and Murray County, Georgia, USA. For each specimen, the head was removed, cut in half, and stored in RNA-later until RNA extraction.

### Molecular data

**Panorpidae transcriptomes** RNA was extracted from the eyes of each adult using a Nucleospin® RNA II column. Approximately 1 μg of RNA was used to construct a cDNA library using Clontech's SMARTer cDNA kit and standard protocol. Following first strand synthesis and cDNA amplification, the cDNA was purified using a Qiagen Qiaquick PCR Purification kit. Purified cDNA was fragmented to ~600 bp via nebulization and further purified using two MinElute PCR Purification kit columns. Ends of the purified, nebulized cDNA were polished and ligated with GS FLX Titanium primers, and small fragment cDNAs were removed via AMPure beads. The ligation was tested via PCR to ensure that the proper adapters were ligated. Following adapter verification, another PCR was done to optimize for the number of cycles to get a consistent visible smear between 300 and 2000 bp on a gel. Once the optimal number of cycles was determined, another round of PCR was done to amplify the

**Table 1** GenBank genes used in BlastX search

| | Query | Source | GenBank |
|---|---|---|---|
| Opsins | Long wavelength | *P. japonica* | AY722619.1 |
| | Ultraviolet | *D. melanogaster* | AH001040.1 |
| | Blue | *B. dorsalis* | AY575956.2 |
| Phototransduction pathway | 26 different genes | Several from *D. melanogaster* | Kegg, ID dme04745l |

**Table 2** Taxa included in phylogenetic analysis along with descriptive information on the opsins generated/obtained from GenBank

| Order | Family | Binomen | Opsin | Length | Start/stop codons | Data source | Accession no. |
|---|---|---|---|---|---|---|---|
| Coleoptera | Tenebrionidae | *Tribolium castaneum* | UVS | 1086 | −/− | GenBank | XM_965251 |
| | | | LWS | 726 | +/+ | GenBank | DQ029113 |
| | Lampyridae | *Luciola cruciata* | LWS | 1143 | +/+ | GenBank | AB300328 |
| | | | UVS | 1149 | +/− | GenBank | AB300329 |
| | Dytiscidae | *Thermonectus marmoratus* | LWS | 1155 | +/+ | GenBank | EU921225 |
| | | | UVS | 1143 | +/+ | GenBank | EU921226 |
| Diptera | Culicidae | *Culex quinquefasciatus* | UVS | 1407 | +/− | GenBank | XM_001861603 |
| | | *Aedes aegypti* | UVS | 1146 | +/+ | GenBank | DQ440445.1 |
| | Therevidae | *Hoplosathe frauenfeldi* | LWS | 452 | −/− | GenBank | AY267595 |
| | Empididae | *Empis* sp. | LWS | 443 | −/− | GenBank | AY267594 |
| | Apsilocephalidae | *Clesthentia* sp. | LWS | 449 | −/− | GenBank | AY267593 |
| | Apsilocephalidae | *Apsilocephala* sp. | LWS | 511 | −/− | GenBank | AY267591 |
| | Hybotidae | *Bicellaria* sp. | LWS | 487 | −/− | GenBank | Y267592 |
| Hymenoptera | Apidae | *Apis cerana* | BS | 1134 | +/+ | GenBank | AB355817 |
| | | | UVS | 1116 | +/+ | GenBank | AB355816.1 |
| | | | LWS | 1134 | +/+ | GenBank | AB355818 |
| | Apidae | *Apis mellifera* | BS | 1134 | +/+ | GenBank | NM_001011606 |
| | | | LWS | 1134 | +/+ | GenBank | NM_001011639 |
| | | | UVS | 1116 | +/+ | GenBank | NM_001011605 |
| | Apidae | *Bombus impatiens* | UVS | 1290 | +/+ | GenBank | AY655163 |
| | Pteromalidae | *Nasonia vitripennis* | LWS | 1911 | +/+ | GenBank | NM_001170908 |
| | Tenthredinidae | *Athalia rosae* | LWS | 1729 | +/− | GenBank | AB437365 |
| Lepidoptera | Nymphalidae | *Heliconius sapho* | BS | 1143 | +/− | GenBank | GU324692 |
| | | *Heliconius doris* | UVS | 1134 | +/+ | GenBank | GQ451897 |
| | | *Eueides lineata* | UVS | 1137 | +/− | GenBank | HM366555 |
| | | *Dione juno* | UVS | 1137 | +/− | GenBank | HM366553 |
| | | *Dryas iulia* | UVS | 1422 | +/− | GenBank | GQ451890 |
| | | *Speyeria mormonia* | LWS | 1716 | +/+ | GenBank | DQ924366 |
| | | *Agraulis vanillae* | LWS | 1488 | +/+ | GenBank | DQ924367 |
| | | *Danaus plexippus* | LWS | 1146 | +/+ | GenBank | AY605545 |
| | | *Heliconius pachinus* | LWS | 1400 | −/− | GenBank | AF126756 |
| | | *Heliconius erato* | LWS | 1143 | +/+ | GenBank | AY918907.1 |
| | | *Euphydryas chalcedona* | LWS | 1561 | +/+ | GenBank | DQ924373.1 |
| | | *Nymphalis antiopa* | LWS | 1612 | +/+ | GenBank | AY740907 |
| | | *Vanessa cardui* | LWS | 1573 | +/+ | GenBank | AF385333 |
| | | *Danaus plexippus* | LWS | 1396 | +/+ | GenBank | AY847475 |
| | | *Neominois redingsii* | LWS | 1533 | +/+ | GenBank | DQ924377 |
| | Riodinidae | *Apodemia mormo* | LWS | 1705 | +/+ | GenBank | AY587907 |
| | Lycaenidae | *Lycaena helloides* | BS | 1016 | +/+ | GenBank | DQ517943 |
| | | | LWS | 1269 | +/+ | GenBank | DQ517949 |
| | | *Lycaena nivalis* | LWS | 1596 | +/+ | GenBank | DQ517951 |
| Mecoptera | Boreidae | *Boreus coloradensis* | BS | 1125 | +/+ | Current Study | |
| | | | LWS | 1140 | + / + | Current Study | |
| | | | UVS | 1128 | +/+ | Current Study | |
| | | *Boreus coloradensis* | LWS | 1391 | −/− | GenBank | AY722614 |
| | Panorpidae | *Panorpa pryeri* | LWS | 981 | −/− | GenBank | AY722617 |
| | | *Panorpa japonica* | LWS | 987 | −/− | GenBank | AY722619 |
| | | *Panorpa lewisi* | LWS | 992 | −/− | GenBank | AY722620 |
| | | *Panorpa nebulosa* | LWS | 995 | −/+ | Current Study | |

**Table 2** (continued)

| Order | Family | Binomen | Opsin | Length | Start/stop codons | Data source | Accession no. |
|---|---|---|---|---|---|---|---|
| | | *Panorpa acuminata* | LWS | 1020 | −/+ | Current Study | |
| | | *Panorpa gracilis* | LWS | 484 | −/− | GenBank | AY722615 |
| | | *Panorpa arakavae* | LWS | 966 | −/− | GenBank | AY722618 |
| Orthoptera | Gryllidae | *Gryllus bimaculatus* | UVS | 1134 | +/+ | GenBank | HM363621.1 |
| Siphonaptera | Pulicidae | *Pulex irritans* | LWS | 1140 | −/− | GenBank | AY722621 |
| | Ctenophthalmidae | *Ctenophthalmus agyrtes* | LWS | 1141 | −/− | GenBank | AY722624 |

cDNA followed by gel purification using a 1 % SeaKem GTG Agarose. From this gel, we used a Caliper LabChip to collect the correct size range prior to loading the samples. The cDNA was then included in an emulsion PCR and prepared for 454 sequencing using the GS FLX Titanium General Library Preparation Method Manual. Transcriptome sequencing was performed on the Roche 454 platform through the BYU DNA Sequencing Center.

*Assembly*: Transcriptomes were assembled using de novo assembly in Newbler (Margulies et al. 2005) under default parameters. Various genes, including opsins, were isolated from the assembled transcriptomes using BLASTN software downloaded from ftp://ftp.ncbi.nih.gov/blast/db. A blastx search was conducted against Panorpid and Drosophila opsins as well as a database of *D. melanogaster* phototransduction genes (Table 1) using a relaxed e-value cut off ($10^{e-5}$). The purpose of these searches was to measure the quality of the transcriptomes.

**Boreidae transcriptome** RNA was extracted from the eye of the boreid using NucleoSpin columns (Clontech) and reverse-transcribed into a cDNA library using the Illumina TruSeq RNA v2 sample preparation that both generates and amplifies full-length cDNAs. The prepped mRNA libraries were sequenced on an Illumina GAiiX in the Brigham Young University sequencing center, Provo, Utah, USA. *Transcriptome assembly*: We performed quality control, assembly, annotation, and transcriptome analysis to facilitate downstream phylogenetic analyses. RNA-seq reads were trimmed using the Mott algorithm implemented in PoPoolation (Kofler et al. 2011), with a minimum read length=40 and quality threshold=20. The de novo assembly of the transcriptome contigs was carried out using Trinity (Grabherr et al. 2011) under the default parameters. *Opsin genes*: Potential light-interacting genes were isolated from each transcriptome by utilizing the Phylogenetically-Informed Annotation (PIA) tool (Speiser et al. 2014; http://galaxy-dev.cnsi.ucsb.edu/pia/), implemented in Galaxy (Giardine et al. 2005; Goecks et al. 2010; Blankenberg et al. 2010). As the PIA tool is optimized to identify an array of light-interacting genes involving circadian cycles, eye development, phototransduction, pigment synthesis, etc., resultant matches in the transcriptomes were then vetted for opsin-specific genes. All individual contigs isolated by the PIA tool were BLASTed, implemented in Geneious®, utilizing the "nr" database option (GenBank, RefSeq, EMBL, DDBJ, and PDB databases), and the "blastn" program set to 100 maximum hits. All non-opsin contigs were ignored, and all putative opsin reads, regardless of length, were mapped in SWISS-MODEL (available from http://swissmodel.expasy.org/) (Arnold et al. 2006; Kiefer et al. 2009; Biasini et al. 2014) to verify the presence of seven trans-membrane regions and aid in the exclusion of partial contigs. A blastx search was conducted against a database of *D. melanogaster* phototransduction genes (download from Kegg, ID dme04745) using a relaxed e-value cut off ($10^{e-5}$).

**Table 3** Transcriptome data for two *Panorpa* species and one *Boreus* species

| Species | Specimen voucher | Sampling location | Sequence method | N raw reads | Assembler | N contigs (>199 bp) | n50 | Longest contig | N contigs (>999 bp) | Total length | N peptide sequences retained |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Boreus coloradensis* (male) | MC 198 | Utah Co, UT, USA | Illumina-GAiiX | 7852291 | Trinity | 25250 | 583.0 | 10641 | 2316 | 12,321,052 | 8345 |
| *Panorpa acuminata* (female) | MC 4 | Forsyth Co, GA, USA | Roche 454 | 171354 | Newbler | 3216 | 1353 | 8427 | 1897 | 4,073,376 | 16555 |
| *Panorpa nebulosa* (female) | MC 9 | Murray Co, GA, USA | Roche 454 | 145331 | Newbler | 1766 | 1197.0 | 4879 | 797 | 1,924,903 | 8215 |

**Table 4** Genes searched for and recovered in two *Panorpa* species and one *Boreus* species

| | *P. acuminata* | *P. nebulosa* | *B. coloradensis* |
|---|---|---|---|
| Genes | | | |
| Long-wavelength R-opsin | √ | √ | √ |
| Ultraviolet R-opsin | – | – | √ |
| Blue R-opsin | – | – | √ |
| Phototransduction pathway (26 genes, total) | √ (25 recovered) | √ (24 recovered) | √ (24 recovered) |

## Phylogenetic reconstruction

Opsin data from other insect taxa were downloaded from GenBank (see Table 2 for accession numbers) and combined with the mecopteran opsins isolated from the transcriptomes for phylogenetic analysis in order to identify opsin classes. Opsin genes were trimmed to the coding sequence (CDS). All opsins were aligned in MAFFT v 7.017 (Katoh and Standley 2013) with the E-INS-i model and checked for open reading frames in MEGA 5.2.2. A Maximum Likelihood analysis was run on the aligned dataset in RAxML (Stamatakis et al. 2008) using the GTR+I+Γ model on the online server (http://embnet.vital-it.ch/raxml-bb/index.php, accessed May 2014). Trees were then visualized in Figtree v. 1.4 (http://tree.bio.ed.ac.uk/software/figtree/).

## Results

The statistics for each assembled transcriptome are shown in Table 3. Using the blast searches, we recovered opsins and phototransduction pathway genes (see Table 4). These results show that the transcriptomes are high enough quality to recover the phototransduction genes that should be present in the eye tissue of the targeted organisms. The LW opsins in all three taxa were recovered, but attempts to recover either blue or UV opsins were successful only for *B. coloradensis*. The absence of blue and UV opsins in Panorpidae is corroborated by previous analyses of panorpid visual systems using electroretinograms (Burkhardt and de la Motte 1972) and Sanger sequencing of cDNA (Taylor et al. 2005).

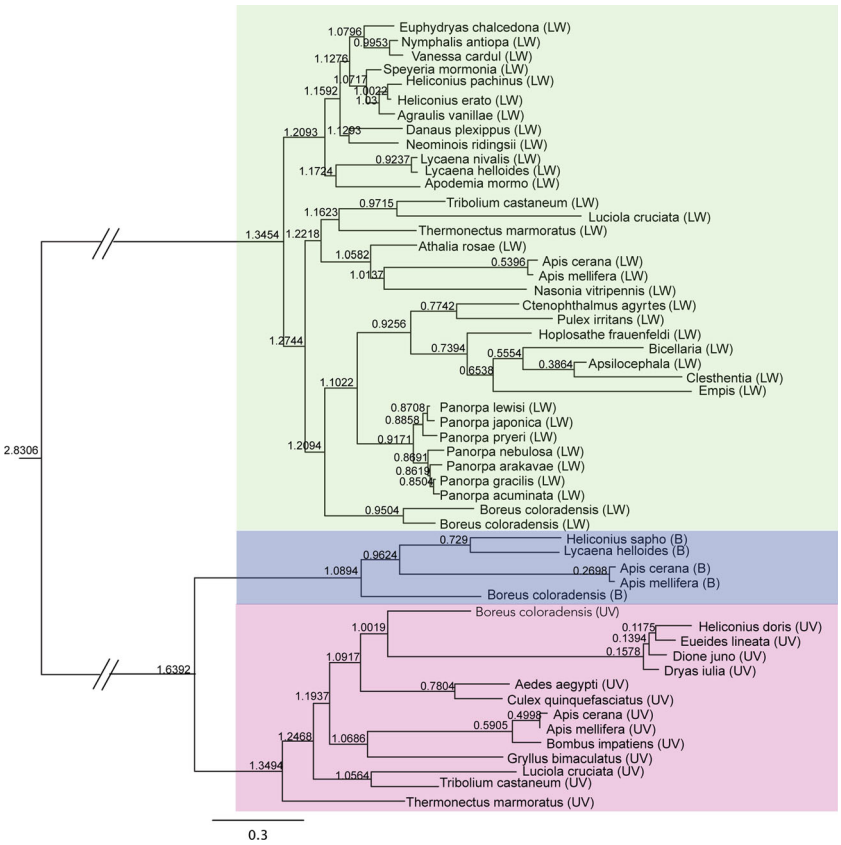


**Fig. 2** Maximum likelihood topology (score of −32468.33) generated from UV, blue, and LW opsin genes (image generated in Adobe Illustrator)

The phylogenetic estimate recovered each opsin class (UV, Blue, and LW) as monophyletic and with high support (Fig. 2). The opsins of *B. coloradensis* were reconstructed within their respective classes (UV, Blue, and LW) and its LW opsin being sister to all other panorpid LW opsins, ours, and those from Taylor et al. (2005), forming a well-supported monophyletic group of mecopteran opsins.

## Discussion

### Role of vision in Panorpidae

We corroborate Burkhardt and de la Motte (1972) and confirm (with molecular data) that adult panorpids express only one LW opsin. Given the highly visual nature of these insects, we were surprised by the recovery of only one LW opsin in each of our panorpid transcriptomes. Recovering all three classes of opsins from the boreid transcriptomes is in line with what we know of the ancestral condition for insect color vision. Although a negative result, the lack of both blue or UV opsins among panorpids is likely accurate and also in line with previous studies (particularly Burkhardt and de la Motte (1972)). These results also do not appear to be caused by poor transcriptome data (Table 3). However, we do acknowledge that both *Panorpa* specimens were female and it is possible that males may express all three copies. Dimorphism in opsin expression is possible (Everett et al. 2012; Ogawa et al. 2012). Burkardt and de la Motte (1972) do not specify the gender of the specimens used in ERG; thus, we cannot verify if he used males or females, only that his experiments only recovered evidence of a single opsin as well.

Presuming there is only one opsin copy, panorpids are likely not capable of color vision, which is intriguing. Wing banding is one of the most diagnostic features of the group and is used in both intra- and interspecific species interactions. This banding is often accompanied by differences in wing coloration and pattern, which varies greatly between species (Fig. 3). It would seem that color discrimination would be required to maximize the contrast between wing banding and wing color within and between species. Although panorpids possess highly visual behaviors, a complex color visual system at the molecular level is not recovered. Our findings in association with others show that panorpid visual systems are likely monochromatic. It may be the contrast between the light and dark bands of their wings that provides the visual cue regardless of wing coloration. If this was the case, they would only need the ability to discriminate between light and dark.

Since the ancestral insect eye likely had all three opsin classes, these results demonstrate a loss of both blue and UV opsin expressions during the evolution of Mecoptera. Panorpids represent a case, among insects with eyes, of monochromy due to loss of opsin expression. These results lead to several interesting questions: (1) Is the expression of blue and UV opsins truly lost in panorpids? (2) Are the underlying genes also lost from the genome? (3) When in the evolution of Mecoptera, did this loss (of expression or from the genome) occur? All are intriguing questions that will likely require transcriptomes and genome-level sequencing of close relatives throughout the family and order, and perhaps even their sister group, Siphonaptera (fleas), to gain a clear perspective for panorpid and mecopteran opsin evolution.

With lacking color vision in the more derived taxa of Panorpidae, we propose that an in-depth look at color vision systems in some of the more basal groups in Panorpidae and of other mecopteran families would be informative. All *Panorpa* that has been examined for opsin expression at the physiological or molecular level (*P. cognata* (Burkhardt and de la Motte 1972) is found in a clade that is sister to *P. nebulosa* and *P. acuminata* (Whiting 2002). While *P. cognata* is a European species, and *P. nebulosa* and *P. acuminata* are from eastern North America (Penny and Byers 1979; Byers 1993), they all are apically positioned in Panorpidae phylogeny and have relatively light wing

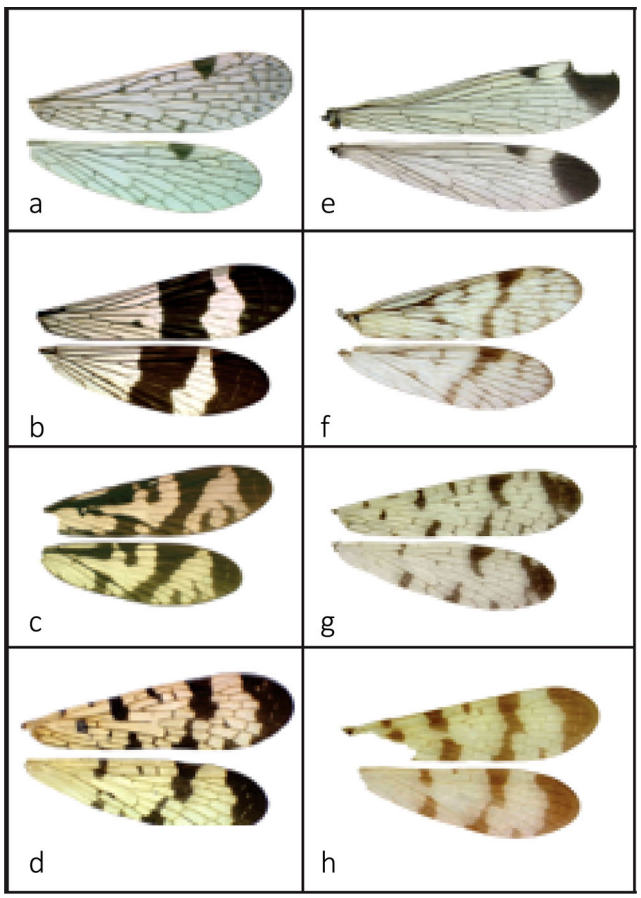


**Fig. 3** Panorpid wing banding in eight taxa. **a** *P. nebulosa*. **b** *P. japonica*. **c** *P. pryeri*. **d** *P. isolata*. **e** *P. lewisi*. **f** *P. arakavae*. **g** *P. gracilis*. **h** *P. acuminata* (chart generated in Adobe Illustrator)

banding relative to the remaining species in the genus. Species such as *P. americana* and *P. nuptialis* have much more intense wing banding and coloration and represent a more basal position among the *Panorpa* species. Exploring the visual systems of these taxa may help answer the question of when the blue and UV opsins were lost in Mecoptera. Our prediction is that the loss of opsin expression occurred in one of three places in the evolution of Mecoptera: (1) Panorpidae+Panorpodidae (very similar lineages), (2) Bittacidae+(Panorpidae+Panorpodidae), or (3) in the base of Panorpidae after *P. nuptialis* (the only panorpid with a big, boldly colored body).

## Future directions

In order to further address the evolution of opsins in Mecoptera, we propose further transcriptome work in addition to genomics. Generating the transcriptomes for other representatives from other Mecoptera families, namely Panorpodidae and Bittacidae, along with more primitive species of Panorpidae will elucidate the evolution of opsin gains/losses in the order. Transcriptomes are more cost effective and less computationally intensive than genomes and can provide similar answers. However, while a broad search of Mecoptera eye transcriptomes is necessary to pinpoint where the loss of opsin copies (or expression of those copies) occurred during the evolution of the group, a genomic-scale analysis is necessary to render a well-defined picture of panorpid visual systems. An entire genome can shed light concerning duplications and the complex evolutionary histories of gene families and provide a broader perspective concerning the evolution of lineages generally (Spaethe and Briscoe 2004; Taylor and Raes 2004; Rivera et al. 2010). With seemingly intricate visually dependent behaviors and diverse wing coloration/banding, it seems astonishing that panorpids may possess the simplest of insect color vision systems. Panorpidae, of all the families of Mecoptera, would seem to be the least likely to possess a single opsin. However, an approach involving transcriptomes and genomes would be able to confirm the presence of only one LW opsin in Panorpidae and explore further questions, such as the gain and loss of other genes involved in phototransduction.

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**Compliance with ethical standards**

**Ethical approval** All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

**Conflict of interest** The authors declare that they have no competing interests.

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