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Geographical color pattern of *Argia apicalis* (Odonata: Coenagrionidae) in the absence of molecular variation

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Tamara J. Cook[3]*, and Jerry L. Cook*[5]

Abstract

The blue-fronted dancer, *Argia apicalis* Say (Odonata: Coenagrionidae), is an ecologically vagile species inhabiting both pond and stream environments of the eastern United States. Variation in color pattern in *A. apicalis* occurs between a southeastern United States morph and a south Florida morph. Southeastern populations often are described as "typical" with a predominantly bright blue pterothorax and narrow black humeral stripe, whereas the southern Florida populations are "atypical," with a bright blue pterothorax and larger, wider black humeral stripes. Variability in color pattern has caused some researchers to question the true identity of the Florida morph. This study used color pattern and mitochondrial cytochrome-b sequences to test the species identity of the 2 *A. apicalis* geographical color morphs*.* Mitochondrial cytochrome-b gene sequences showed that there is a single haplotype, showing no divergence between individuals, populations, or regions. This study is the first to test if color pattern variation is correlated with molecular characters within this species.

Key Words: damselflies; insect; distribution; cytochrome-b

Resumen

El caballito del diablo bailarín de frente azul, *Argia apicalis* Say (Odonata: Coenagrionidae), es una especie ecológicamente vágil que habita el ambiente de lagunas y de quebradas del este de los Estados Unidos. La variación en el patrón de color en *A. apicalis* se produce entre un morfo sureste de los Estados Unidos y un morfo del sur de la Florida. Se describen las poblaciones del sudeste a menudo como "típica" con un pterotórax azul predominantemente brillante y con una franja del húmero estrecha del color negro, mientras que las poblaciones del sur de la Florida son "atípicos", con un pterotórax azul brillante y rayas negras de húmero más grandes y más anchas. La variabilidad en el patrón de color ha hecho que algunos investigadores cuestionen la verdadera identidad del morfo de la Florida. Este estudio utilizó el patrón de color y las secuencias de citocromo-b mitocondrial para probar la identidad de las 2 formas geográficas de morfos de *A. apicalis.* Las secuencias de genes de citocromo-b mitocondrial mostraron que hay un solo haplotipo, sin mostrar divergencia entre individuos, poblaciones o regiones. Este estudio es el primero en probar si la variación del modelo de color se correlaciona con caracteres moleculares dentro de esta especie.

Palabras Clave: caballitos del diablo; insecto; distribución; citocromo-b

With only a few exceptions, odonates do not exhibit much intraspecific color pattern variation that is clearly correlated with geography despite distributions that encompass several ecological niches and wide areas. A noted exception is the *Argia fumipennis* (Odonata: Coenagrionidae) complex found in the southeastern region of the United States, which is composed of 3 sub-species: *A. fumipennis fumipennis* (Burmeister), *A. fumipennis violacea* (Hagen), and *A. fumipennis atra* Gloyd, and the subspecies are distinguished based only on wing color and distribution (Burmeister 1839; Hagen 1861; Gloyd 1968). *Argia apicalis* (Say) (Coenagrionidae: Odonata) is a species complex that shows a similar geographical color pattern variation.

Argia apicalis is an ecologically vagile species inhabiting both pond and stream environments. Say (1839) diagnosed *A. apicalis* as having a pearlaceous blue thoracic region and a hairline humeral and thin dorsal stripe (Fig. 1A). Other characters used to identify *A. apicalis* are the distinctive pointed and tooth-like cercus in males (Fig. 1B) and a distribution east of the Rocky Mountains and into Arizona (Fig. 1C) (Garrison 1994).

Bick & Bick (1965), Dunkle (1990), Johnson & Westfall (1970), and Johnson (1972) noted variation of the humeral stripe in southeastern populations of *A. apicalis*. The subpopulation of *A. apicalis* in the southeastern range (Suwannee County and Columbia County, Florida) have a broad humeral stripe across the length of the pterothorax. In his analysis of the geographical variation within *A. apicalis* in Florida, Johnson (1972) categorized both males and females into groupings based on the amount of black markings on the head, thorax, and abdomen, and defined "typical" *A. apicalis* as having humeral stripe values of 1 or 2, whereas "atypical" specimens had values of 4 or 5. Specimens with a value of 3 were deemed intermediates. Johnson (1972) then discussed the pattern variability in the southeastern distribution and noted that in this region specimens with the typical pattern were less than 5% and specimens with atypical thoracic patterns represented more than 95% of the population. In the summer of 2013, 2 authors herein (Smith-Herron & T. Cook) collected *A. apicalis* near the Suwannee River and noted that 100% of their specimens (122 specimens) fit Johnson's defi-

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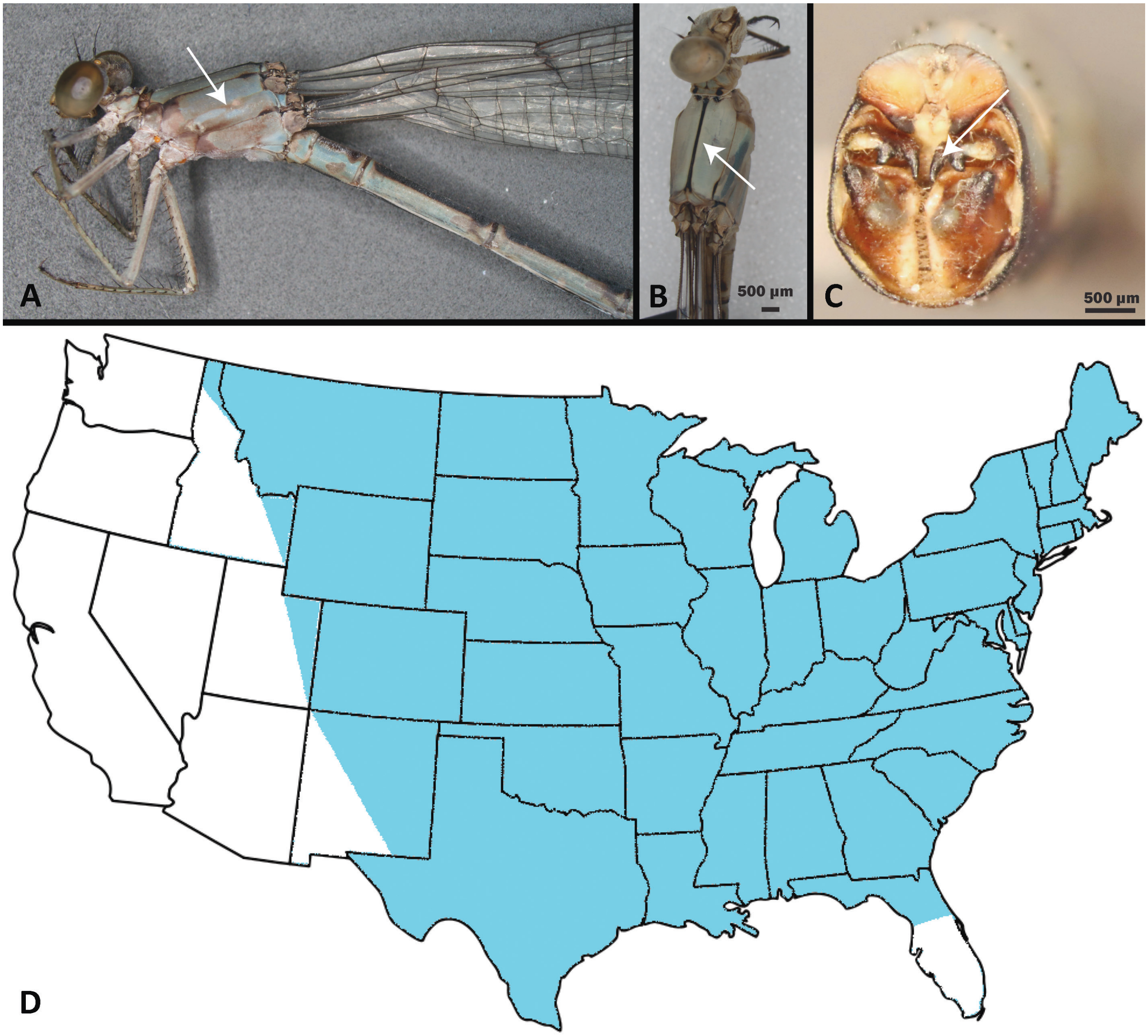

Fig. 1. The 4 defining characters of *Argia apicalis*; **(A)** a pearlaceous blue pterothorax and a hairline humeral stripe; **(B) a** thin dorsal stripe; **(C)** males have a distinctive pointed and tooth-like cercus; **(D)** and their distribution east of the Rocky Mountains (shaded areas on map are the recorded distribution of *A. apicalis*).

nition of atypical. This study is the first to combine color pattern and cytochrome-b gene sequences to document variation within an extensive portion of the distribution range of *A. apicalis.*

Materials and Methods

INSECT COLLECTIONS

Argia apicalis adults were collected using aerial nets in May through Sep 2013 and 2014 from 10 localities in Texas, Louisiana, Florida, and Oklahoma (Table 1). Individuals were field preserved in 70 to 90% ethanol and subsequently dried and curated upon arrival at the laboratory. Specimens were photographed with a Canon EOS 70D mounted on an Olympus SZX12 microscope.

We also obtained about 200 specimens on loan from the following museum collections: National Museum of Natural History (NMNH), International Odonata Research Institute (IORI), Georgia Museum of Natural History (GMNH), and Sam Houston State Entomology Collection (SHSUEC), which allowed us to augment our coverage of the distributional range of *A. apicalis*. The combination of our field-collected samples with existing museum specimens represent about 59% of the reported distribution of *A. apicalis* in the United States (Fig. 2) (Westfall & May 2006).

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Table 1. List of *Argia apicalis* specimens examined for color pattern analysis.

Museum accession numbers	Date	Sex	State	County/Parish	Locality
USMN 00354813	1 VIII 1914	F	DC	N/A	C&O canal, Chain Bridge
USMN 00354821	22 VIII 1974	M	FL	Jackson	N/A
USMN 00354822	22 VIII 1974	F	FL	Jackson	N/A
IORI 00038262	10 VI 1954	—	FL	Liberty	Torreya State Park
IORI	15 VIII 1969	M	FL	Holmes	Choctowhatchee River at US 90
IORI	12 IV 1975	F	FL	n/a	Escambia River
IORI	25 VIII 1950	M	FL	Columbia	Santa Fe River
IORI	12 VII 1972	M	FL	Clay	Black Creek, N. Prong, Hwy 209
IORI	2 VIII 1969	M	FL	Suwannee	Suwannee River, Suwannee State Park
IORI	11 VIII 1969	M	FL	Wakulla	St. Marks River at Newport on US 98
IORI	28 V 1973	M	FL	Gadsden	Near Apalachicola River, Hwy 90, Chattahoochee
IORI	28 V 1973	F	FL	Gadsden	Near Apalachicola River, Hwy 90, Chattahoochee
IORI	22 VIII 1984	F	FL	Jackson	Three Rivers State Park
IORI	12 VIII 1969	M	FL	Liberty	Ochlockonee River at Hwy 20
IORI	7 V 1981	—	FL	Washington	Choctowhatchee River at US 90
IORI	33 VIII 1984	M	FL	Jackson	Three Rivers State Park
IORI	12 VII 1972	F	FL	Clay	Black Creek, N. Prong , Hwy 209
IORI	29 IV 1973	M	FL	Columbia	Pond near Santa Fe River, Hwy 441
IORI	18 IX 1971	F	FL	Alachua	Santa Fe River, near Hwy 235
USMN 00354826	20 VIII 1946	M/F	GA	Decatur	Spring Creek (mating pair)
GMNH	10 VII 1955	—	GA	Morgan	N/A
GMNH	7 VII 1971	—	GA	Clarke	N/A
GMNH	23 VI 1950	—	GA	Sumter	N/A
GMNH	20 V 1984	—	GA	Clarke	N/A
GMNH	13 VI 1950	—	GA	Sumter	N/A
GMNH	22–27 VII 1970	—	GA	Clarke	N/A
GMNH	20 VI 1938	—	GA	Clarke	N/A
GMNH	20 VI 1937	—	GA	Clarke	N/A
GMNH	26 V 1953	—	GA	Clarke	N/A
GMNH	9–13 V 1994	—	GA	Clarke	1.1 mile SW of Winterville
GMNH	VI 1937	—	GA	Clarke	N/A
GMNH	2 VII 1944	—	GA	Fulton	Bolton
GMNH	27 VII 1944	—	GA	Fulton/Dekalb	Atlanta
GMNH	2 VII 1944	—	GA	Fulton	Bolton
GMNH	25 VII 1941	—	GA	Putnam	Eatonton
GMNH	24 VII 1931	—	GA	Whitfield	Dalton
GMNH	16 VI 1946	—	GA	Decatur	Spring Creek
USMN 00354841	24 VII 1929	M	IL	Lake	4-5 mi. N. of Olney
USMN 00354842	24 VII 1929	F	IL	Lake	4-5 mi. N. of Olney
USMN 00355162	26 VII 1953	M	IN	Tippecanoe	Wabash River, N. of Lafayette
USMN 00355164	26 VII 1953	M	IN	Tippecanoe	Wabash River, N. of Lafayette
USMN 00354873	8 VII 1927	M	IA	N/A	Iowa River; SUI Campus
USMN 00354875	15 VII 1927	F	IA	N/A	Iowa River; SUI Campus
USMN 00354900	10 VII 1943	M	KS	Sumner	Caldwell
USMN 00354901	10 VII 1943	F	KS	Sumner	Caldwell
USMN 00354908	18 VI 1947	F	KY	Green	Crailhope; Little Barren River
USMN 00354906	29 VII 1948	M	KY	Green	Crailhope; Little Barren River
USMN 00354909	12 VIII 1925	F	LA	Madison	Eagle Lake
USMN 00354910	12 VIII 1925	M	LA	Madison	Eagle Lake
USMN 00354930	21–28 VII 1903	M	MD	Montgomery	Barnesville; C&O canal
USMN 00354928	21–28 VII 1903	F	MD	Montgomery	Barnesville; C&O canal
USMN 00354969	16 VII 1907	M	MN	Washington	Stillwater
USMN 00354970	16 VII 1907	F	MN	Washington	Stillwater
USMN 00354981	28 VIII 1949	F	MO	Oregon	N/A
USMN 00354979	28 VIII 1949	M	MO	Oregon	N/A
GMNH	16 IX 1972	—	MO	Boone	N/A
USMN 00355192	20 VII 1998	M	NB	Cherry	Along Niobrara R; Allen Bridge; 4 mi. S of Sparks
USMN 00355192	20 VII 1998	M	NB	Cherry	Along Niobrara R; Allen Bridge; 4 mi. S of Sparks
USMN 00355200	3 VIII 1985	F	NJ	Hunterdon	Lockatong Cr. On Hwy 29 N; Stockton
USMN 00355204	3 VIII 1985	M	NJ	Hunterdon	Lockatong Cr. On Hwy 29 N; Stockton
USMN 00355209	19 IX 1988	M	NM	Guadalupe	Santa Rosa

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Table 1. (Continued) List of *Argia apicalis* specimens examined for color pattern analysis.

Museum accession numbers	Date	Sex	State	County/Parish	Locality
USMN 00355213	18 VIII 1939	F	NC	Swain/Jackson	Cherokee; Hiwassee River; Murphy
USMN 00355214	18 VIII 1939	M	NC	Swain/Jackson	Cherokee; Hiwassee River; Murphy
USMN 00355218	20 VII 1926	F	OH	Erie	Huron R. ; 5 mi. S. of Huron
USMN 00355222	20 VII 1926	M	OH	Erie	Huron R. ; 5 mi. S. of Huron
USMN 00355247	1962	F	OK	Paine	N/A
USMN 00355248	1960	M	Ok	Garfield	N/A
USMN 00355254	13–14 VI 1970	M	PA	York	Conewago Cr.; 5 mi. NW of Davidsburg
USMN 00355257	18 VII 1939	M	SC	Greenville	Lakeside; a lake approx. 7 mi. S. of Greenville
USMN 00355256	18 VII 1939	F	SC	Greenville	Lakeside; a lake approx. 7 mi. S. of Greenville
USMN 00355275	14 VIII 1939	F	TN	Campbell	Lafollette; Cove Lake
USMN 00355272	31 V 1953	M	TN	Davidson	Nashville; pool in Centennial Park
USMN 00355297	7 IX 1949	M	TX	Cameron	Brownsville
USMN 00355303	15–17 VI 1965	M	TX	San Jacinto	Near Coldspring
USMN 00355304	8 VI 1965	F	TX	Wharton	El Campo
USMN 00355309	26 VI 1904	M/F	TX	n/a	Dallas (mating pair)
USMN 00355306	15–17 VI 1965	M	TX	San Jacinto	Near Coldspring
USMN 00391416	12–26 VII 2007	F	VA	Fairfax	Quarry; Great Falls; 39.984722 -77.250278
USMN 00391417	12–26 VII 2007	M	VA	Fairfax	Swamp Trail; Great Falls; 38.984444 -77..250556
USMN 00713680	13 VI 2007	F	WV	Marshall	Dunkard; Fork Lake; 1.2 mi. S. of Majorsville off CR 15
USMN 00713676	24 VIII 2004	M	WV	Harrison	Good Hope; West Fork River
SHSUE 026337	20 VII 2013	M	FL	Suwannee	Suwannee State Park along the river
SHSUE 026336	20 VII 2013	M	FL	Suwannee	Suwannee State Park along the river
SHSUE 026339	20 VII 2013	F	FL	Suwannee	Suwannee State Park along the river
SHSUE 026338	20 VII 2013	M	FL	Suwannee	Suwannee State Park along the river
SHSUE 026335	20 VII 2013	F	FL	Suwannee	Suwannee State Park along the river
SHSUE 026344	20 VII 2013	F	FL	Suwannee	Suwannee State Park along the river
SHSUE 026345	20 VII 2013	M	FL	Suwannee	Suwannee State Park along the river
SHSUE 026349	31 VIII 2013	F	TX	Taylor	Jim Ned Creek
SHSUE 026348	31 VIII 2013	F	TX	Taylor	Jim Ned Creek
SHSUE 026346	31 VIII 2013	M	TX	Taylor	Jim Ned Creek
SHSUE 000185	1 IX 2007	—	OK	Tulsa	Mohawk State Park
SHSUE 000161	23 VII 2007	—	TX	Brown	Yegua Creek
SHSUE 000189	10 VIII 2007	—	TX	Parker	Mineral Wells
SHSUE 001293	25 VII 2012	—	TX	Walker	Raspberry Pond, Phelps, TX
SHSUE 000173	1 VII 2007	—	NE	Omaha	Easley Creek
SHSUE 026343	20 VII 2013	M	FL	Suwannee	Jim Ned Creek
SHSUE 026340	19 IX 2013	M	OK	Waynoka	Waynoka Stream
SHSUE 000195	2 IX 2007	—	KS	Sedewick	Pawnee Prairie
SHSUE 000162	2 VII 2007	—	KS	Brown	Delaware River
SHSUE 008488	12 VI 2013	—	LA	Calcacien	Sam Houston Jones State Park
SHSUE 008519	17 VI 2013	—	LA	Washington	Bogue Chitto Stream
SHSUE 000692	1 VII 2011	—	TX	Taylor	Jim Ned Creek
SHSUE 026347	31 VIII 2013	F	TX	Taylor	Jim Ned Creek
SHSUE 026341	19 IX 2013	F	OK	Waynoka	Waynoka Stream
SHSUE 026342	19 IX 2013	F	OK	Waynoka	Waynoka Stream
SHSUE 008592	20 VII 2013	—	FL	Suwannee	Suwannee State River Park
SHSUE 008599	20 VII 2013	—	FL	Suwannee	Suwannee State River Park
SHSUE 008601	20 VII 2013	—	FL	Suwannee	Suwannee State River Park
SHSUE 008600	20 VII 2013	—	FL	Suwannee	Suwannee State River Park
SHSUE 008602	20 VII 2013	—	FL	Suwannee	Suwannee State River Park
SHSUE 008603	20 VII 2013	—	FL	Suwannee	Suwannee State River Park
SHSUE 008604	20 VII 2013	—	FL	Suwannee	Suwannee State River Park
SHSUE 008579	20 VII 2013	—	FL	Suwannee	Suwannee State River Park
SHSUE 008580	20 VII 2013	—	FL	Suwannee	Suwannee State River Park
SHSUE 008581	20 VII 2013	—	FL	Suwannee	Suwannee State River Park
SHSUE 008582	20 VII 2013	—	FL	Suwannee	Suwannee State River Park
SHSUE 008583	20 VII 2013	—	FL	Suwannee	Suwannee State River Park
SHSUE 008585	20 VII 2013	—	FL	Suwannee	Suwannee State River Park
SHSUE 008586	20 VII 2013	—	FL	Suwannee	Suwannee State River Park
SHSUE 014225	24 VII 2013	—	TX	Liberty	Big Thicket: Birdwatcher's Trail
SHSUE 014226	24 VII 2013	—	TX	Liberty	Big Thicket: Birdwatcher's Trail

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Table 1. (Continued) List of *Argia apicalis* specimens examined for color pattern analysis.

Museum accession numbers	Date	Sex	State	County/Parish	Locality
SHSUE 026437	20 V 2014	—	TX	Brewster	Rio Grande River
SHSUE 026438	20 V 2014	—	TX	Brewster	Rio Grande River
SHSUE 007459	20 V 2014	—	TX	Brewster	Rio Grande River
SHSUE 007460	20 V 2014	—	TX	Brewster	Rio Grande River
SHSUE 007461	20 V 2014	—	TX	Brewster	Rio Grande River
SHSUE 007462	20 V 2014	—	TX	Brewster	Rio Grande River

MORPHOLOGICAL CHARACTERS

Since its description in 1839, researchers have used hairline humeral stripe found on the thorax to identify and distinguish *A. apicalis* from its congeners (Johnson 1972). We examined the width of the humeral stripe of the thorax and the pattern and coloration of the head and thorax. For the humeral stripe, the width of the stripe, along the entire length of the thorax, was evaluated and whether or how much it narrowed along its length (Fig. 3). Johnson (1972) reported that individuals with large black patterning on the head usually had hairline humeral stripes whereas individuals with small patterning had wider humeral stripes. To test this observation, we examined head and abdomen of specimens for distinct patterns between the southeastern and the northwestern populations. We also evaluated variation in the morphology of the male caudal appendages and female mesostigmal plates (Garrison 1994; Westfall & May 2006).

MOLECULAR ANALYSES

We extracted genomic DNA from legs for a subset of 29 individuals (Table 2) following the standard protocol instructions of Zymo Research's Quick-gDNA™ Mini Prep extraction kit (Zymo Research, Irvine, California). Individuals selected for DNA extraction were taken from field-collected specimens as most museum specimens included in the morphological analyses were collected over 20 yr ago. Special care was taken to select individuals from populations throughout the geographic distribution of *A. apicalis* (i.e., Florida, Kansas, Louisiana, Oklahoma, and Texas). Although the mitochondrial cytochrome oxidase I (COI) gene has been used widely to detect cryptic diversity and inter-population differentiation in odonates (Brown et al. 2000) and other hexapods (Folmer et al. 1994; Marcus et al. 2009), repeated attempts to amplify this gene fragment in our samples using various previously published primers [e.g. HCO/LCO (Folmer et al. 1994), EVA/JERRY (Blum et al. 2003)] failed to produce positive amplicons. We thus decided to PCR amplify a 361 bp fragment of the mitochondrial gene cytochrome-b (*cyt-b*) using primers (151F: 5'-TGTGGRGCNACYGTW-3'; 270R: 5'-AANAGGAARTAYCAYTCNGGYTG-3') and conditions published by Merritt et al. (1998). The usefulness of this gene fragment in detecting cryptic diversity and population structure in insects and other arthropods is well established (Simmons & Weller 2001; Santamaria et al. 2013). Positive amplicons were cleaned and sequenced at Genewiz (South Plainfield, New Jersey), with resulting sequences assembled and edited (e.g., removal of primer regions) using Geneious R 8.0.2 (Biomatters Ltd.). Dried specimens from which DNA was extracted were labeled as DNA vouchers and deposited into the SHSUEC. DNA template vouchers are stored at −40 °C in the Sam Houston State Natural History Collections (SHSNHC) housed at the Texas Research Institute for Environmental Studies (TRIES) facility, Sam Houston State University.

Results

ANALYSIS OF COLOR PATTERN

Specimens from the northwestern distribution of *A. apicalis* (north of the Florida Panhandle) displayed a "typical" hair-line humeral stripe. The humeral stripe in this distribution range varied from almost ab-

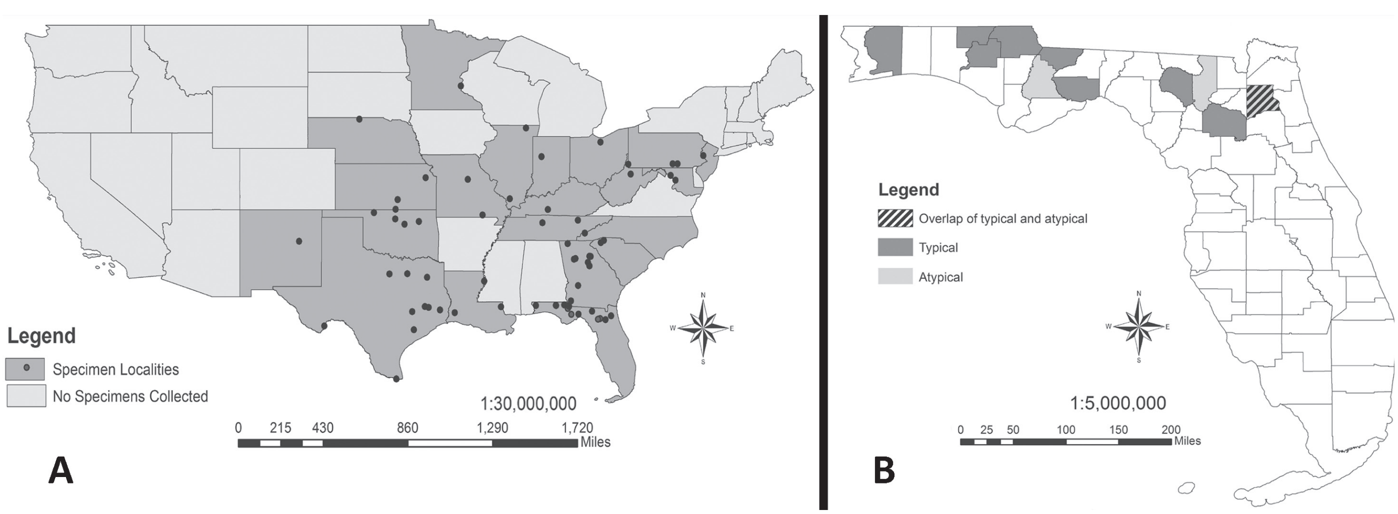

Fig. 2. **(A)** Distribution map of specimens examined (this accounts for about 59% of the reported distribution of *Argia apicalis*). Dark gray states with black dots represent collected specimen localities, and light gray states with no dots represent areas where no specimens were collected. **(B)** Distribution of color morphs of *A. apicalis* in Florida; dark gray represents counties with typical *A. apicalis*, light gray represents counties with atypical *A. apicalis*, and the striped area represents the county in which both typical and atypical *A. apicalis* morphs are present.

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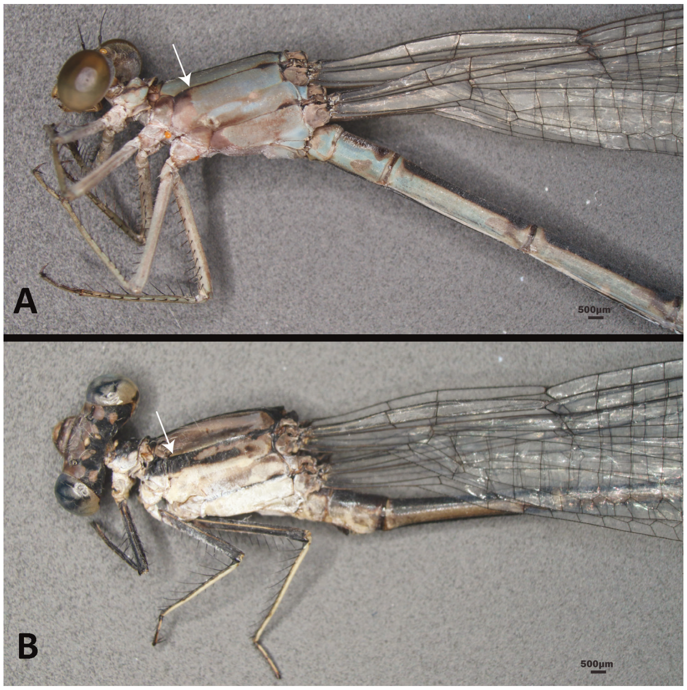

Fig. 3. Geographical color variation: individuals from the **(A)** northwestern range of the distribution have a typical (= narrow) humeral stripe, whereas individuals from the **(B)** southeastern part of the range have an atypical (= wide) humeral stripe.

sent in some specimens to very narrow at most, and rarely extended more than a third of the length of the pterothorax. Although the thickness of the humeral stripe varied in the northwestern populations, the variation was not as pronounced as that between northwestern and southeastern populations (Figs. 4–6). Southeastern populations had an "atypical" humeral stripe that was much wider than the "typical" variation and generally extended beyond the anterior third of the pterothorax and might extend the entire length of the pterothorax. Patterns on the head and abdomen were variable among all individuals and were not correlated with geography. After examining all specimens, we noted that the black patterns on the head and abdomen were variable across all specimens and all geographic regions.

MOLECULAR ANALYSIS

All sequences produced in this study were deposited in GenBank under accession numbers KP770147 to KP770165. We successfully sequenced the target *cyt-b* gene fragment for 19 *A. apicalis* individuals from throughout its range in the United States: 8 from 5 populations in Texas, 7 from a single population in Florida, 3 from 2 populations in Oklahoma, and 1 from a single population in Kansas. All 19 individuals harbored a single haplotype for the *cyt-b* gene, indicating no divergence between individuals, populations, or regions. The remaining 11 DNA extractions (4 from Texas, 1 from Florida, 1 from Oklahoma, 2 from Louisiana, 2 from Kansas, and 1 from Nebraska) failed to produce positive amplicons despite repeated attempts at PCR amplification and re-extraction of DNA. Given our findings of non-existent genetic divergence in this gene, we decided against further efforts to produce sequences for these samples.

Table 2. List of *Argia apicalis* specimens processed for molecular analysis.

Museum accession numbers	Date	Sex	State	County/Parish	Locality
SHSUE 026337	20 VII 2013	M	FL	Suwannee	Suwannee State River Park
SHSUE 026336	20 VII 2013	M	FL	Suwannee	Suwannee State River Park
SHSUE 026339	20 VII 2013	F	FL	Suwannee	Suwannee State River Park
SHSUE 026338	20 VII 2013	M	FL	Suwannee	Suwannee State River Park
SHSUE 026335	20 VII 2013	F	FL	Suwannee	Suwannee State River Park
SHSUE 026344	20 VII 2013	F	FL	Suwannee	Suwannee State River Park
SHSUE 026345	20 VII 2013	M	FL	Suwannee	Suwannee State River Park
SHSUE 026349	31 VIII 2013	F	TX	Taylor	Jim Ned Creek
SHSUE 026348	31 VIII 2013	F	TX	Taylor	Jim Ned Creek
SHSUE 026346	31 VIII 2013	M	TX	Taylor	Jim Ned Creek
SHSUE 000185	1 IX 2007	—	OK	Tulsa	Mohawk State Park
SHSUE 000161	23 VII 2007	—	TX	Brown	Yegua Creek
SHSUE 000189	10 VIII 2007	—	TX	Parker	Mineral Wells
SHSUE 001293	25 VII 2012	—	TX	Walker	Raspberry Pond, Phelps, TX
SHSUE 000173	1 VII 2007	—	NE	Omaha	Easley Creek
SHSUE 026343	20 VII 2013	M	FL	Suwannee	Jim Ned Creek
SHSUE 026340	19 IX 2013	M	OK	Waynoka	Waynoka Stream
SHSUE 000195	2 IX 2007	—	KS	Sedewick	Pawnee Prairie
SHSUE 000162	2 VII 2007	—	KS	Brown	Delaware River
SHSUE 008488	12 VI 2013	—	LA	Calcacien	Sam Houston Jones State Park
SHSUE 008519	17 VI 2013	—	LA	Washington	Bogue Chitto Stream
SHSUE 000692	1 VII 2011	—	TX	Taylor	Jim Ned Creek
SHSUE 026347	31 VIII 2013	F	TX	Taylor	Jim Ned Creek
SHSUE 026341	19 IX 2013	F	OK	Waynoka	Waynoka Stream
SHSUE 026342	19 IX 2013	F	OK	Waynoka	Waynoka Stream
SHSUE 007459	20 V 2014	—	TX	Brewster	Rio Grande River
SHSUE 007460	20 V 2014	—	TX	Brewster	Rio Grande River
SHSUE 007461	20 V 2014	—	TX	Brewster	Rio Grande River
SHSUE 007462	20 V 2014	—	TX	Brewster	Rio Grande River

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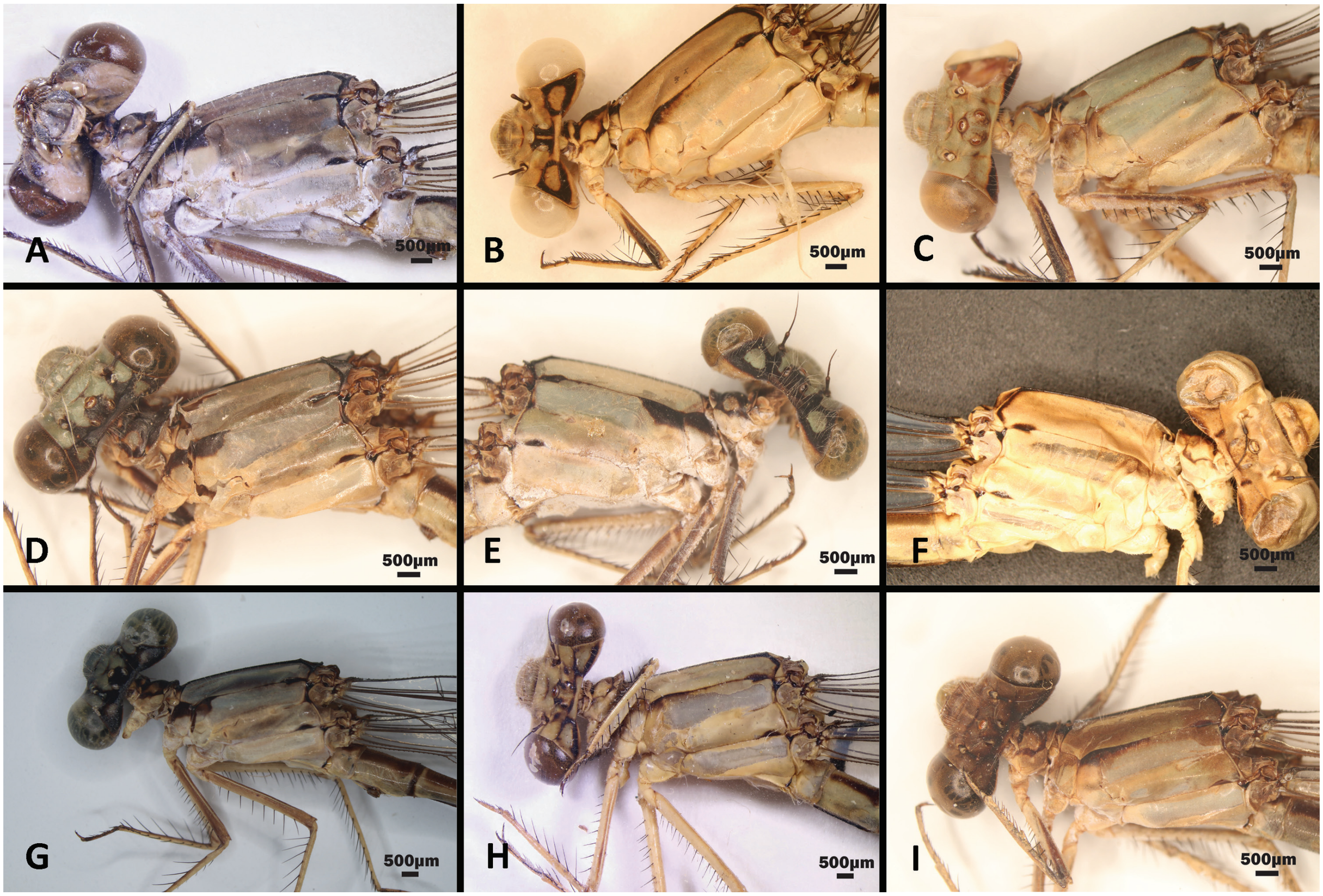

Fig. 4. Variation in width and extension of humeral stripes in the northwest region of the distribution: **(A)** Dallas County, Texas; **(B)** Fairfax County, Virginia; **(C)** Hunterdon County, New Jersey; **(D)** Iowa; **(E)** Missouri County, Oregon; **(F)** Washington Parish, Louisiana; **(G)** Holmes County, Florida; **(H)** Wharton County, Texas; and **(I)** Washington D.C.

Discussion

The 2 color forms of *A. apicalis* were described briefly by Johnson (1972) and Dunkle (1990). Descriptions of *A. apicalis* have characterized it as having a pterothorax with a narrow dorsal stripe and either no or at most thin humeral stripe, except for populations from Florida in which the humeral stripe is wider and extends more than half the length of the pterothorax. The geographical divide of the 2 color forms is the Suwannee River and reflects the population disjunctions of the sub-species of *A. fumipennis* in which *A. f. atra* occurs east of the Suwannee River, *A. f. fumipennis* west of the Suwannee River, and *A. f. violacea* north/northwest of Florida. Where the color morphs overlap, they are intermediate in the color pattern and cannot be identified further.

The 2 color forms of *A. apicalis* were once documented to co-exist near the Suwannee River but their co-existence may no longer be true. As we found no differences in *cyt-b* gene sequences between the 2 color morphs throughout their distribution range, their recognition as separate species is not justified. Although no mechanism or cause is known for the variation seen in both the *A. fumipennis* and *A. apicalis* complexes, Johnson (1972) suggested allopatric processes associated with sea level changes during the Pleistocene (e.g., fragmentation of Florida into islands and formation of the Suwannee Straits) may be responsible for the observed differences.

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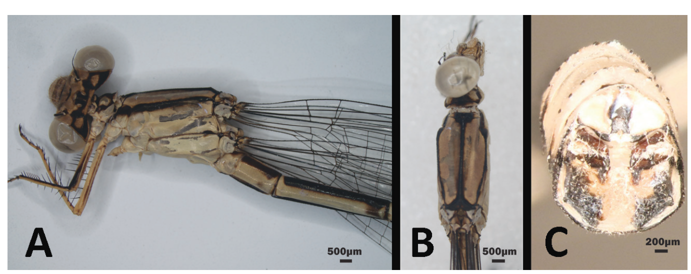

Fig. 5. Identifying characters of *Argia apicalis* in the southeast: **(A)** humeral stripe wide extending at least three-quarters of the pterothorax length; **(B)** mid-dorsal line slightly wider than in northwestern *A. apicalis*; and **(C)** paler caudal appendage (whiter) than in individuals from the north.

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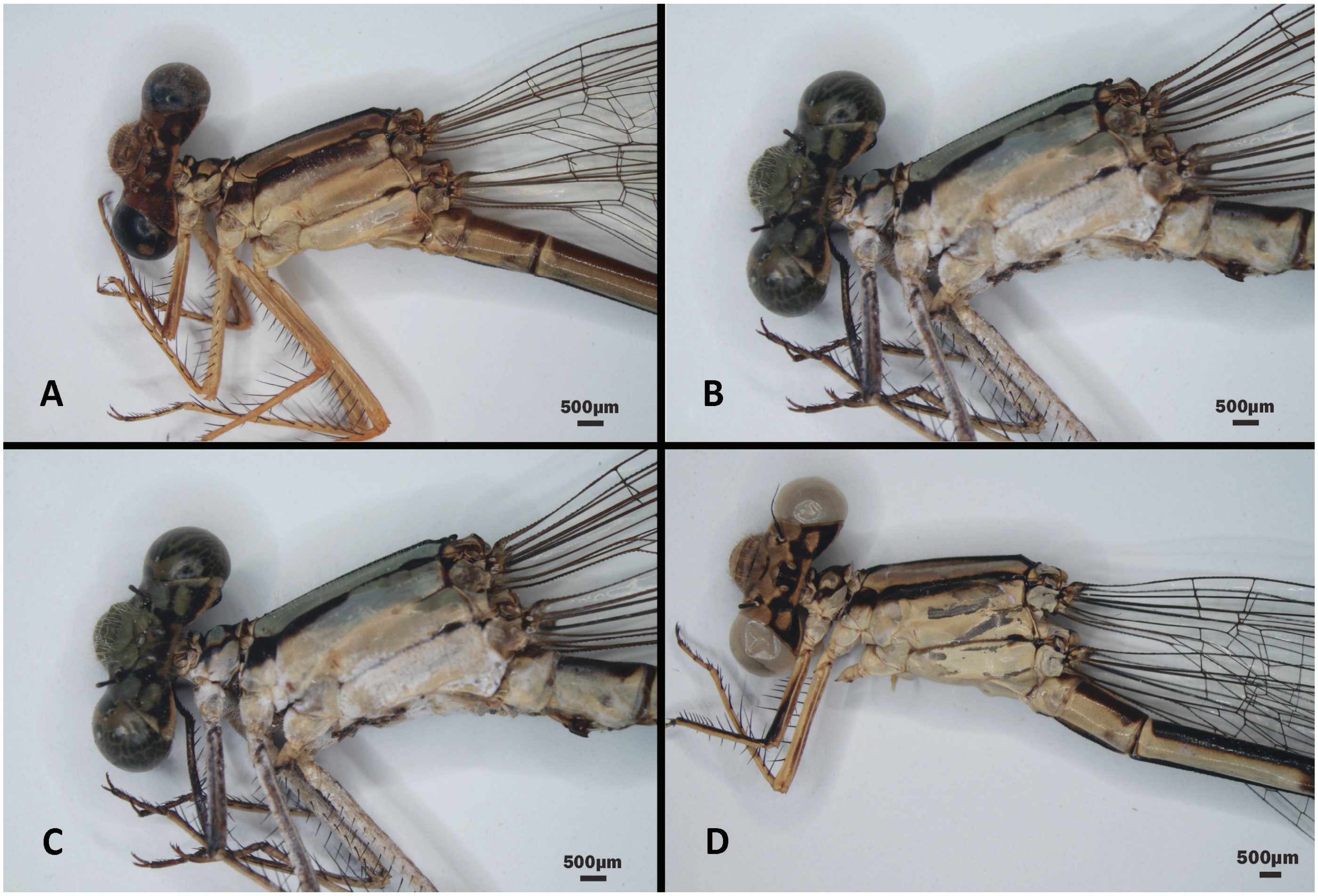

Fig. 6. Variation of humeral stripes in the southeast region of the distribution: **(A)** Clay County, Florida; **(B)** Columbia County; Florida; **(C)** Wakulla County, Florida; and **(D)** Suwannee County, Florida.

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