

**A New Species of *Crossodactylodes* from the Espinhaço Mountain Range, Southeastern
Brazil (Anura: Leptodactylidae: Paratelmatobiinae)**

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RRH: SANTOS ET AL.—A NEW SPECIES OF *CROSSODACTYLODES*

ABSTRACT: The bromeligenous genus *Crossodactylodes*, endemic to the Atlantic Forest

domain and the “campo rupestre” ecosystem in Brazil, currently comprises five named species. Three additional putatively new species have already been proposed in a recent study based on mitochondrial and nuclear markers. Here we employ phenotypic data to corroborate the distinctiveness of one of these lineages, and describe it as a new species, from the Espinhaço Mountain Range in the municipality of Itamarandiba, state of Minas Gerais, southeastern Brazil. We also provide information on its natural history and conservation status. The new species is diagnosable from its congeners by a combination of characters, including the presence of vocal slits in adult males, the orange coloration of discs on fingers and toes, and the presence of vomerine odontophores. The new species is the second *Crossodactylodes* to be reported for the “campo rupestre” and it was only recorded in a small forest patch composed of low trees, shrubs, mosses, lichens, and a high density of bromeliads.

Key words: Amphibia; Bromeligenous; Campo rupestre; Species description; Taxonomy

THE GENUS *Crossodactylodes* Cochran 1938, one of the four genera of the Brazilian endemic subfamily Paratelmatobiinae Ohler and Dubois 2012, currently comprises five species: *C. pinto* Cochran 1938; *C. bokermanni* Peixoto 1983 “1982”; *C. izecksohni* Peixoto 1983 “1982”; *C. septentrionalis* Teixeira, Recoder, Amaro, Damasceno, Cassimiro, and Rodrigues 2013; and *C. itambe* Barata, Santos, Leite and Garcia 2013. The genus is supported by DNA sequence data, as well as by several putative synapomorphies, including a life cycle strictly dependent on bromeliads (bromeligenous habit); the nuptial pads formed by few and well-developed keratinized spines; the tympanic middle ear completely lacking; and the presence of expanded discs on fingers II–IV (Fouquet et al. 2013; Santos et al. 2020a,b). Additionally, the genus is characterized by a small body size (snout–vent length < 20 mm) and by producing

complex but low-intensity acoustic signals (Santos et al. 2020a, 2022).

Species of *Crossodactylodes* are disjunctly distributed across the mountains within the Atlantic Forest domain and the “campo rupestre” ecosystem (i.e., rupestrian grasslands; Miola et al. 2021), from southern Bahia to the state of Rio de Janeiro (Santos et al. 2020b). With the exception of *C. bokermanni*, which is known from four nearby sites in the municipality of Santa Teresa and two other localities in the state of Espírito Santo, all other species are currently known only from one or a few nearby localities (Santos et al. 2020a). Factors such as the small geographic range, the occurrence in remote areas, the use of specific microhabitats, the small body size, the reduced vocal activity, and the production of low-intensity calls hinder the sampling of new populations, indicating that the species diversity of *Crossodactylodes* is likely underestimated (Santos et al. 2020a, 2022). Indeed, recent surveys in different mountainous areas have resulted in the discovery of new populations and three of them were previously proposed as putatively new species based on nuclear and mitochondrial markers (Santos et al. 2020b; Fig. 1).

We formally describe one of these putatively new species, the *Crossodactylodes* sp. 3 of Santos et al. (2020b), from the Espinhaço Mountain Range in the municipality of Itamarandiba, state of Minas Gerais, southeastern Brazil. The new species represents the second member of the genus to be reported from the “campo rupestre.” Our description is based on distinct morphological characters in comparison with congeners, adding to the previous evidence from DNA sequence data. We also provide information on the natural history and the conservation status of the new species.

MATERIALS AND METHODS

Field Data and Specimens Collection

We conducted fieldwork from 26–28 April 2017 and from 28 February to 4 March 2018,

at Parque Estadual da Serra Negra, a protected area located in the municipality of Itamarandiba, Brazilian state of Minas Gerais, in the Espinhaço Mountain Range. Specimens were collected manually or with the aid of mouth-operated aspirators (Silva and Alves-Silva 2008) from ground and epiphytic bromeliads beside the “Barro Preto” trail (18°00’39.19”S, 42°44’30.34”W, 1,556 m above sea level; datum = WGS84), in a small forest patch composed of low trees, shrubs, mosses, lichens, and a high density of bromeliads.

The collected specimens were euthanized in 5% lidocaine solution, fixed in 10% commercial grade formalin, and preserved in 70% ethanol. Type specimens are housed in the Célio F.B. Haddad Amphibian Collection, Rio Claro, São Paulo, Brazil (CFBH); Amphibian Collection of Centro de Coleções Taxonômicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil (UFMG); Amphibian Collection of Museu Nacional, Rio de Janeiro, Rio de Janeiro, Brazil (MNRJ); and Herpetological Collection of Museu de Zoologia da Universidade de São Paulo, São Paulo, São Paulo, Brazil (MZUSP).

Morphological Analyses

Terminology for morphological characters follows Duellman (1970), Heyer et al. (1990), Duellman and Lehr (2009), and Santos et al. (2020a). Fingers are numbered preaxially to postaxially from I to IV. Terminology for nuptial pad follows Luna et al. (2018), and that for egg morphology and ovipositional mode follows Altig and McDiarmid (2007). Measurements follow Duellman (1970) for snout-vent length (SVL), head length (HL), head width (HW), eye diameter (ED), tibia length (TBL), and foot length (FL); Heyer et al. (1990) for hand length (HAL), thigh length (THL), and tarsal length (TAL); Napoli (2005) for eye-nostril distance (END), nostril to tip of snout distance (NSD), third finger disk diameter (3FD) and fourth toe disk diameter (4TD); Garcia et al. (2003) for distance between the anterior margins of eyes (AMD); Cei (1980) for internarial distance (IND); Duellman et al. (1997) for forearm length (FAL); and Greene and

Funk (2009) for arm length (AL), arm width (AW), and forearm width (FAW). The measurements were taken to the nearest 0.1 mm under a stereomicroscope, using a digital caliper for SVL, HL, HW, AL, FAL, HAL, THL, TBL, TAL, and FL; and an ocular micrometer for ED, END, NSD, IND, AMD, AW, FAW, 3FD, and 4TD. All measurements were taken by M.T.T. Santos.

Description of coloration in life is based on field observations and photographs. Sex of specimens was determined by the presence of secondary sexual characteristics in adult males (i.e., vocal slits, nuptial pads, and forelimb hypertrophy) and by the presence of mature oocytes in females. As we found specimens with few spines on nuptial pads but lacking discernable vocal slits, we comparatively dissected the gular region of some specimens to confirm the absence/presence of vocal slits (i.e., large males bearing vocal slits on both sides of the buccal floor and several spines on nuptial pads: CFBH 46177, 46183; and small males with few spines on nuptial pads either lacking discernable vocal slits: CFBH 44889, 46180, or having it just on one side of the buccal floor: MZUSP 159319). We also compared the degree of development of testes in some of these specimens (CFBH 46180, 46183). We considered a male specimen as an adult when it had vocal slits present on both sides of the buccal floor (but see the Remarks section).

For taxonomic comparisons, we examined specimens including type material of all congeneric species (Appendix). Museum acronyms follow Sabaj (2020). Due to the lack of knowledge of the coloration in life of *C. pinto* and to the poor preservation of coloration on collection specimens, we did not consider characters related to color pattern in comparisons with this species.

SPECIES DESCRIPTION

Crossodactylodes serranegra sp. nov.

(Table 1; Figs. 2–4)

Holotype.—Adult male (CFBH 44887; field number MTTS 480) collected at “Barro Preto” trail, Parque Estadual da Serra Negra, municipality of Itamarandiba, state of Minas Gerais, southeastern Brazil (18°0’39.2”S, 42°44’30.3”W, 1,556 m above sea level), on 26 February 2018, by M.T.T. Santos, I.M. Barata, and S.V. Martins (Figs. 2, 3).

Paratopotypes.—10 adult males (CFBH 44890, 46177, 46182–84, 46187, 46189; MNRJ 93960; MZUSP 159317, 159320), four subadult males (CFBH 44889, 46180; MNRJ 93958; MZUSP 159319), 10 adult females (CFBH 44888, 46176, 46178–79, 46181, 46185–86, 46188, 46190; MZUSP 159318), one subadult female (MNRJ 93959), collected with the holotype on 26 February to 04 March 2018. Two adult males (UFMG 19823–24) collected on 26–28 April 2017 by I.M. Barata, M.A. Becheleni, and A.C. Ferreira.

Diagnosis.—*Crossodactylodes serranegra* is diagnosable from its congeners by the following combination of characters: (1) adult males with vocal slits; (2) in life, discs on fingers and toes orange; (3) presence of vomerine odontophores; (4) inner metacarpal tubercle weakly widened; (5) in life, iris coppery with dark brown fine reticulations; (6) snout not flattened in lateral view; (7) absence of dorsolateral fold; (8) discs of fingers II–IV slightly expanded; (9) skin on males dorsum coarsely granular; (10) disc of Finger I rounded; (11) medial region of the upper lip not anteriorly projected; (12) females SVL 15.3–16.4 mm; (13) karyotype with $2N = 32$ chromosomes.

Comparisons.—*Crossodactylodes serranegra* is distinguished from its congeners (characters in parentheses) by the presence of vocal slits in adult males (absence in *C. itambe*, *C. izecksohni*, *C. pinto*, and *C. septentrionalis*); discs on fingers and toes in life with orange coloration (without orange coloration in *C. itambe*, *C. izecksohni*, and *C. septentrionalis*);

presence of vomerine odontophores (absence in *C. itambe*, *C. izecksohni*, and *C. pinto*); inner metacarpal tubercle in adult males weakly widened, i.e., in ventral view it is not wider than Finger I (broadly widened, tubercle clearly wider than Finger I, in *C. izecksohni* and *C. septentrionalis*); iris in life coppery with dark brown fine reticulations (yellowish with dark brown fine reticulations, interrupted by a brown horizontal bar at the pupil level in *C. izecksohni* and uniformly black or brownish black in *C. itambe*); snout not flattened in lateral view (flattened in *C. bokermanni* and *C. septentrionalis*). Additionally, *C. serranegra* can be distinguished from *C. bokermanni* by the absence of dorsolateral fold (presence); discs of fingers II–IV slightly expanded (broadly expanded); skin on male dorsum coarsely granular (shagreen). From *C. septentrionalis* by having disc of Finger I rounded (acute). From *C. itambe* by having the medial region of the upper lip not anteriorly projected (anteriorly projected). From *C. izecksohni* by having larger females, with SVL 15.3–16.4 mm (females SVL 10.8–13.9 mm). It also differs from *C. itambe* and *C. izecksohni*, the only other species with known karyotypes, by having $2N = 32$ chromosomes ($2N = 36$ in *C. itambe*; $2N = 30$ in *C. izecksohni*; see Pedroso et al. 2022).

Description of holotype.—Adult male, SVL 17.1 mm; body slightly robust, dorsoventrally flattened; head slightly wider than long (HW/HL 1.05); head width 0.36 SVL; head length 0.35 SVL; snout not flattened, rounded in dorsal view, sloping in lateral view; END slightly longer than ED (END/ED 1.13); canthus rostralis slightly curved in dorsal view and rounded in cross-section; loreal region slightly concave; nostrils protuberant, elliptical, dorsolaterally directed; interorbital area flat, more than twice as long as ED (AMD/ED 2.51). Eyes medium-sized and protuberant (ED/HL 0.23; ED/HW 0.22), laterally oriented; upper eyelid margin granular, with an elongated flat tubercle in its medial region. Tympanic membrane and annulus absent. Poorly developed dermal fold from posterior edge of eye curving downward to arm insertion. Dorsolateral fold absent.

Choanae small, nearly rounded, spaced 1.5 mm from each other. Vomerine odontophores present, positioned posterior to the level of the choanae. Tongue ovoid, not notched, free behind for about one-third of its length. Vocal slits short, located at the level of the posterior third of tongue; vocal sac externally indistinct. Single, small tooth-like process present anteriorly on lower jaw, fitting to a socket between premaxillae.

Forelimb robust, slightly hypertrophied, lacking fold or fringe; forearm slightly less robust than arm (FAW/AW 0.92); fingers medium-sized; relative length of fingers $I < II \approx IV < III$; discs of fingers II–IV slightly expanded, rounded; disc of Finger I rounded, less expanded than the others; fingers II–IV slender; Finger I bearing a nuptial pad formed by a conspicuous cluster of dark colored spine-shaped papillae covering dorsomedially the skin of Metacarpus I and prepollex (eight spines on left and seven on right Finger I); subarticular tubercles small, oval in ventral view on Finger I and round in ventral view on fingers II–IV; supernumerary tubercles slightly distinct; inner metacarpal tubercle flat, elliptical, narrower than Finger I in ventral view; outer metacarpal tubercle large, flat, nearly round in ventral view, with a poorly developed indentation on the distal margin; vestigial digital webbing.

Hindlimb medium-sized and moderately robust (THL/SVL 0.42; TBL/SVL 0.39); tarsal fold absent; toes long, slender, with slightly expanded and nearly rounded discs, slightly larger than finger discs (4TD/3FD 1.06); relative length of toes $I < II < V < III < IV$; subarticular tubercles small, flat, oval in ventral view on Toe I, and rounded in ventral view on Toes II–V; supernumerary tubercles slightly distinct; inner metatarsal tubercle large, flat, and elliptical in ventral view; outer metatarsal tubercle small, nearly round in ventral view, slightly conical in profile; digital webbing absent.

Skin coarsely granular except on ventral surfaces of hindlimbs, throat, and pectoral region (shagreen). Cloacal opening directed posteriorly at upper level of thighs, covered by a cloacal

flap with a small distal indentation.

Measurements of holotype (in mm).—SVL 17.1; HL 5.9; HW 6.2; ED 1.4; END 1.5; NSD 0.9; IND 1.4; AMD 3.4; AL 4.0; AW 1.9; FAL 3.2; FAW 1.7; HAL 4.2; 3FD 0.7; THL 7.3; TBL 6.7; TAL 4.0; FL 6.2; 4TD 0.7.

Coloration.—In life, dorsum light brown with dark brown blotches and scattered bluish white flecks. A dark brown interorbital blotch. A dark brown M-shaped blotch from the scapular region to the medial third of the body. A dark brown, rounded blotch in the lumbar region. A barely distinguishable dark brown bar in the wrist. Hindlimbs with well-marked dark brown bars. A barely distinguishable dark brown canthal band and lateral band from the posterior corner of the eye to the arm insertion (Fig. 2A). Venter light brown with scattered whitish flecks (Fig. 2B). Discs on fingers and toes, upper eyelid tubercle, and outer metatarsal tubercle orange. Iris copper-colored with dark brown fine reticulations. Spines on nuptial pads black. During daylight the coloration is darker (Fig. 2C). In preservative, coloration is similar to live specimens in daylight. The blotches, except those on hindlimbs, become indistinct. The orange colors of fingers, toes, and tubercles fade and become cream (Fig. 3).

Variation.—The cloacal flap usually has a small distal indentation (approx. 71% of specimens), but can be bilobed (approx. 25%), and is rarely undivided (approx. 4%). The tubercle on medial region of upper eyelid margin is almost always present (approx. 93%) but can be absent on one (approx. 3.5%) or both (approx. 3.5%) sides. The tubercle can be poorly (approx. 61.5%) or well-developed (approx. 38.5%). It usually has an elongated shape on both eyelids (approx. 73%) but can be elongated on one eyelid and rounded on the other (approx. 27%).

Males can be distinguished from females by the presence of nuptial pads, by having a rougher dorsal skin, and a greater hypertrophy of forelimbs (males AW/AL 0.42–0.64, mean 0.52; females AW/AL 0.27–0.36, mean 0.32; and males FAW/FAL 0.54–0.75, mean 0.66;

females FAW/FAL 0.37–0.47, mean 0.43). The spine-shaped papillae on nuptial pads vary in size, degree of aggregation, and number (from two, in the subadult male MZUSP 159319, to 13 in the adult CFBH 46183). The number of spines can vary between left and right Finger I of a same specimen. In general, larger males have a more pronounced hypertrophy of forelimbs, a rougher skin, and a higher number of spines on nuptial pads.

Coloration pattern in life exhibited some variation (Fig. 4). The number and size of blotches vary from almost no blotch (Fig. 4A, D), a few small blotches (Fig. 4E), a few large blotches (Fig. 4B), or several large blotches (Fig. 4C, F). Specimens may have a few (Fig. 4B), several (Fig. 4F), or many (Fig. 4C) whitish flecks. The canthal band vary from barely distinguishable (Fig. 4B, D, E) to well-marked (Fig. 4C, F), but can be absent (Fig. 4A). The lateral band is usually absent (Fig. 4A, B, D) or inconspicuous (Fig. 4E), but can be well-marked, extending from the posterior corner of the eye to the arm insertion (Fig. 4C) or to the inguinal region (Fig. 4F).

Etymology.—The specific epithet *serranegra* is a Portuguese word that means black mountain, in reference to the type locality. The epithet is used as a noun in apposition.

Call.—The call was described by Santos et al. (2022) as *Crossodactylodes* sp.

Cytogenetics.—*Crossodactylodes serranegra* has a diploid number of $2N = 32$. The karyotype was described in detail by Pedroso et al. (2022) as *Crossodactylodes* sp. 3.

Distribution.—*Crossodactylodes serranegra* is known only from its type locality at Parque Estadual da Serra Negra, 18°0'39.2"S, 42°44'30.3"W, 1,556 m above sea level, municipality of Itamarandiba, in the state of Minas Gerais, southeastern Brazil (Fig. 5).

Habitat and natural history.—*Crossodactylodes serranegra* was found in a small forest patch (approx. 13 ha) composed of low trees, shrubs, mosses, lichens, and a high density of bromeliads in the “campo rupestre” ecosystem, at the eastern border of the Central Espinhaço

Mountain Range (Fig. 6). At the locality, average bromeliad tank size was 88 cm² (height × width), mean water depth was 3.4 cm, and average water temperature inside the tank was 19°C ($n = 63$). Although we searched other forest fragments as well as open areas at Parque Estadual da Serra Negra (approximately 65 bromeliads repeatedly surveyed for three consecutive nights) we did not find specimens elsewhere but the type locality. The new species was recorded in epiphytic and terrestrial *Vriesea schwackeana* and *V. longicaulis*, which are the most abundant species of bromeliads in the forest fragment. There are other species of bromeliads in the area (e.g., *Vriesea bituminosa* and *Neoregelia* sp.), but no specimens of *C. serranegra* were found in them (Fig. 6).

Specimens were found alone or in aggregations (i.e., occupying a same bromeliad). These aggregations included: (1) one male, two females, one tadpole, and one egg; (2) one male, two tadpoles, and one egg; (3) one male and two juveniles; (4) one male and one female; (5) one female and one tadpole. Females lay single large eggs, positioned isolated from other eggs, attached to bromeliad leaves and above the water surface. Eggs are uniformly darkly pigmented.

Conservation.—*Crossodactylodes serranegra* is known to occur only at Parque Estadual da Serra Negra, a strictly protected area (IUCN Category II; Dudley 2008) of 13,654.31 ha. The species was only found in a small forest patch. Although the forest fragment is approximately 13 ha, we only searched an area of approximately 0.5 ha. Thus, following the IUCN criteria the current known area of occupancy is approximately 4 km² (IUCN 2022). Species could occur more widely within the fragment. The main threats to the species are arson and collection of bromeliads, which might be reducing the breeding habitat of *C. serranegra*. Although the species occurs in a strictly protected area, the available data on geographic distribution, ecological requirements, and possible threats are insufficient to classify it into a threat category. Thus, we suggest *C. serranegra* to be categorized as Data Deficient.

Remarks.—Santos et al. (2022) reported vocal slits to be absent in *C. serranegra* (as

Crossodactylodes sp.). However, after a detailed analysis of a larger sample, we found some specimens lacking vocal slits or having it just on one side of the buccal floor, generally with a smaller SVL, a lower hypertrophy of forelimbs, a lower number of spines on nuptial pads, and smaller testes (which we considered as subadult males); and others bearing vocal slits and generally having a greater SVL, a higher hypertrophy of forelimbs, a higher number of spines on nuptial pads, and greater testes (which we considered as adults); Fig. 7.

There are few studies addressing the timing of differentiation and development of secondary sexual characters such as nuptial pads and vocal slits during post-metamorphic ontogeny of anurans and their relationship with gonadal and reproductive maturation (Olmstead et al. 2009; Goldberg et al. 2016). In *C. serranegra*, it is clear that nuptial pads develop much earlier than vocal slits during ontogeny, but this cannot be interpreted as direct evidence of sexual maturity. For instance, males of *Xenopus tropicalis* exhibit nuptial pads eight weeks after metamorphosis, but only reach reproductive maturation after 22 weeks (Olmstead et al. 2009). For vocal slits, the timing of development is well known only for a few taxa and can originate shortly after metamorphosis (e.g., *Pseudis minuta*; Goldberg et al. 2016) or may arise lately in adult life (e.g., some species of *Craugastor*; Zweifel 1956). Future studies aiming to clarify the ontogenetic testicular development in *Crossodactylodes* as well as observations of breeding behavior (i.e., if males without vocal slits are able to mate) are important to correctly elucidate the period of sexual maturity of males in the genus. Therefore, we acknowledge that our definition of subadult/adult specimens is somewhat restrictive and might change with further evidence.

Noteworthy, one male specimen of *C. serranegra* (CFBH 44889) included in the study of Santos et al. (2022) did not have vocal slits but was recorded in calling activity (CFBH-voc 4; Santos et al. 2022). No acoustic differences were found in comparison with the male that had

vocal slits (CFBH 44890). Moreover, males of species that lack vocal slits (*C. itambe* and *C. izecksohni*) were also reported in calling activity and there are no clear differences between the calls that would be attributed to the absence/presence of vocal slits. Considering the relatively small size of the vocal sac of *C. serranegra* and *C. bokermanni* and its absence in the other species, as well as the limited vocal activity, the production of low-intensity calls, and the absence of a tympanic middle ear reported for the genus (Santos et al. 2020a; this study) it seems likely that the absence/presence of a vocal sac does not have major implications for sound production in the genus.

DISCUSSION

The genus *Crossodactylodes* remained monotypic for over 45 years, until the description of two new species by Peixoto (1983 “1982”). Three decades later, the number of species increased to five when two additional species were described (Barata et al. 2013; Teixeira et al. 2013). This 30-yr gap is likely a result of uneven sampling effort combined with the species’ cryptic life history traits. Firstly, there is a strong spatial bias in data collection in Brazil (Oliveira et al. 2016) and amphibian survey effort is unequally distributed within the southern Espinhaço Range (Barata et al. 2016). Secondly, besides the very reduced geographic ranges of the species of *Crossodactylodes*, factors such as the occurrence in very specific microhabitats, the inconspicuous behavior, and the reduced vocal activity with low-intensity calls hamper the discovery of as-yet unknown populations and species. Despite the sampling difficulties, recent studies focused on the genus have resulted in a better understanding of its evolutionary relationships, natural history, and ecology (Barata et al. 2017, 2018; Ferreira et al. 2019; Santos et al. 2017, 2020a,b, 2022). One of these studies detected three putatively new species of *Crossodactylodes* (Santos et al. 2020b) and the formal description of *C. serranegra* represents a

step further in addressing the Linnean shortfall in the genus (Hortal et al. 2015; Pante et al. 2015).

As knowledge has advanced, the habitat requirements for the species of *Crossodactyloides* have become clearer. The species are strictly associated with bromeliads on humid mountaintops in both Atlantic Forest and “campo rupestre.” Unknown populations and species in the genus are likely to be discovered, mainly in under-sampled remote mountain ranges (Santos et al. 2020a,b). A careful inspection of bromeliads using a wide range of survey methods during herpetological inventories will likely increase the chance of detection and collection of specimens of *Crossodactyloides*. For instance, the discovery of *C. serranegra* is a result of targeted cost-effective surveys based on distribution models that were used to pinpoint areas of high suitability for plant and frog occurrence (I.M. Barata, personal communication). During field work it is common to observe specimens of *Crossodactyloides* diving very quickly and hiding at the bottom of bromeliad axils in response to disturbance (Santos et al. 2017; Ferreira et al. 2019). Additionally, species detection in the field can vary between observers, increasing significantly with survey experience (Barata et al. 2017). Finally, the flattened shape of their bodies hinders the manual collection of specimens. For these reasons, the use of mouth-operated aspirators substantially increases the chance of collecting specimens. The use of environmental DNA (eDNA) can also improve the detection of phytotelm species (Barata et al. 2021; Mullin et al. 2022), such as bromeliad dwelling frogs. Barata et al. (2021) demonstrated that *C. itambe* can have a high detection rate (0.97–0.98) by extracting and amplifying eDNA from water samples as little as 120 ml.

The type locality of *C. serranegra* in the Parque Estadual da Serra Negra, eastern Espinhaço Mountain Range, also harbors other microendemic species such as the plants *Comanthera brunnea* and *Eugenia salacifolia* (Echternacht and Sano 2012; Bünger et al. 2018). The locality is still poorly sampled and other unknown endemic species possibly occur in the

region. The finding of *C. serranegra*, the second species in the genus to be reported for the “campo rupestre”, reinforces that this ecosystem played a key role in the diversification of the genus. It is somewhat unexpected that the two taxa occurring in the “campo rupestre” are not sister species (Fig. 1; Santos et al. 2020b) and, possibly, a complex evolutionary scenario involving multiple colonizations and/or extinctions may have driven the diversification of *Crossodactylodes*.

The “campo rupestre” is considered an old, climatically buffered, infertile landscape (OCBIL; see Hopper 2009 and Silveira et al. 2016), harboring a high number of ancient evolutionary endemic lineages (Silveira et al. 2016). This is the case with the two species of *Crossodactylodes* (i.e., *C. itambe* and *C. serranegra*), which were tentatively inferred to have diversified during the Miocene (Santos et al. 2020b). According to Hopper (2009), the biota of OCBILs is associated with very specific habitat types, generally has a reduced dispersal rate that favors allopatric speciation, and may exhibit genetic, cytogenetic, or phenotypic adaptations that conserve heterozygosity in the face of inbreeding due to small population sizes. The chromosome number variation reported for *Crossodactylodes*, with the “campo rupestre” endemic species having the highest diploid numbers (Pedroso et al. 2022), is congruent with the predictions of the OCBIL theory. Whether these chromosome changes were involved in speciation events in the genus by acting, for instance, as a pre-zygotic isolation mechanism or were related to stochastic events due to a long-term isolation deserves further investigation.

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SUPPLEMENTAL MATERIAL

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APPENDIX

Specimens Examined

Crossodactylodes bokermanni.—BRAZIL: ESPÍRITO SANTO: municipality of Santa Teresa, Alto Santo Antônio, type locality (paratypes: EI 7174–76, 7180–83, 7189; topotypes: ZUFRJ 1377–78, 1380, 1382, 1933–34); Reserva Biológica Augusto Ruschi (CFBH 44754); Penha district (CFBH 44748–53; UFMG 14189).

Crossodactylodes itambe.—BRAZIL: MINAS GERAIS: municipality of Santo Antônio do Itambé, Parque Estadual do Pico do Itambé (holotype: UFMG 11239; paratypes: UFMG 11236–38, 11240–47, 11249, 13375–85, 13387; topotypes: CFBH 44651, 45186–87; UFMG 14031–40).

Crossodactylodes izecksohni.—BRAZIL: ESPÍRITO SANTO: municipality of Santa Teresa, Alto Santo Antônio, type locality (paratypes: EI 7193–7209; topotypes: UFMG 397, 399, 13747; ZUFRJ 361, 365, 1392, 1400 1932, 1935, 1401); another locality in Alto Santo Antônio (UFMG 14174–86, 14214–17, 14219–21); hill in front of Escola Superior São Francisco de Assis (UFMG 13748–49, 13753, 14029–30); vicinity of Reserva Biológica Augusto Ruschi (UFMG 14222–24).

Crossodactylodes pinto.—BRAZIL: RIO DE JANEIRO: municipality of Nova Friburgo, Serra de Macaé (holotype: MZUSP 56467; paratypes: MZUSP 104; USNM 102608).

Crossodactylodes septentrionalis.—BRAZIL: BAHIA: municipality of Arataca, Parque Nacional da Serra das Lontras (holotype: MZUSP 150209; topotypes: MZUESC 14363–64).

TABLE 1.—Measurements and proportions for the adult specimens of the type series of *Crossodactylodes serranegra*. Values (mm) are reported as ranges (mean \pm SD). See text for measurement abbreviations.

	Measurement			Body ratios	
	Males ($n = 13$)	Females ($n = 10$)		Males ($n = 13$)	Females ($n = 10$)
SVL	15.2–18.9 (17.1 \pm 1.2)	15.3–16.4 (15.9 \pm 0.4)	HW / HL	1.02–1.11	1.01–1.07
HL	5.4–6.4 (6.0 \pm 0.3)	5.4–6.0 (5.7 \pm 0.2)	HL / SVL	0.34–0.37	0.35–0.37
HW	5.8–6.8 (6.3 \pm 0.4)	5.7–6.2 (5.9 \pm 0.2)	HW / SVL	0.36–0.39	0.36–0.39
ED	1.3–1.5 (1.4 \pm 0.1)	1.3–1.4 (1.4 \pm 0.03)	END / ED	0.98–1.14	0.96–1.11
END	1.4–1.6 (1.5 \pm 0.1)	1.4–1.5 (1.4 \pm 0.04)	AMD / ED	2.36–2.63	2.24–2.57
NSD	0.9–1.1 (0.9 \pm 0.1)	0.8–0.9 (0.9 \pm 0.04)	AMD / HL	0.56–0.62	0.54–0.62
IND	1.4–1.6 (1.4 \pm 0.1)	1.3–1.5 (1.4 \pm 0.1)	AMD / HW	0.53–0.59	0.53–0.58
AMD	3.2–3.9 (3.6 \pm 0.2)	3.2–3.5 (3.3 \pm 0.1)	ED / HL	0.23–0.26	0.23–0.25
AL	3.5–4.5 (4.2 \pm 0.3)	3.4–3.9 (3.6 \pm 0.1)	ED / HW	0.21–0.24	0.22–0.24
AW	1.5–2.9 (2.2 \pm 0.4)	1.0–1.4 (1.1 \pm 0.1)	AL / SVL	0.23–0.26	0.21–0.24
FAL	2.9–3.4 (3.3 \pm 0.1)	3.0–3.3 (3.1 \pm 0.1)	AW / AL	0.42–0.64	0.27–0.36
FAW	1.7–2.5 (2.2 \pm 0.3)	1.2–1.4 (1.3 \pm 0.1)	FAL / SVL	0.18–0.21	0.18–0.21

HAL	3.9–4.5 (4.2 ± 0.2)	3.6–4.0 (3.7 ± 0.1)	FAW / FAL	0.54–0.75	0.37–0.47
3FD	0.6–0.8 (0.7 ± 0.05)	0.6–0.7 (0.6 ± 0.03)	HAL / SVL	0.22–0.27	0.23–0.25
THL	6.5–7.7 (7.2 ± 0.3)	6.4–7.0 (6.6 ± 0.2)	3FD / HAL	0.16–0.18	0.16–0.19
TBL	6.1–6.8 (6.6 ± 0.2)	5.7–6.2 (6.0 ± 0.1)	THL / SVL	0.40–0.45	0.41–0.43
TAL	3.7–4.2 (3.9 ± 0.2)	3.4–3.7 (3.6 ± 0.1)	TBL / SVL	0.36–0.41	0.37–0.39
FL	5.4–6.4 (6.0 ± 0.3)	4.7–5.9 (5.4 ± 0.3)	TAL / SVL	0.21–0.25	0.22–0.24
4TD	0.7–0.8 (0.7 ± 0.04)	0.6–0.7 (0.7 ± 0.03)	FL / SVL	0.32–0.38	0.31–0.37
			4TD / FL	0.12–0.14	0.11–0.14
			4TD / 3FD	1.00–1.12	1.00–1.13

FIGURE CAPTIONS

FIG. 1.—A simplified phylogenetic tree (50% majority rule consensus from Bayesian inference), showing the relationships within the genus *Crossodactylodes* (modified from Santos et al. 2020b). The analysis was based on a matrix comprising three mitochondrial (heavy strand transcription unit 1, which includes 12S and 16S rRNA, plus the intervening tRNA^{valine}; cytochrome c oxidase subunit I; and cytochrome b) and five nuclear (proopiomelanocortin A; recombination-activating protein 1; rhodopsin; tensin 3; and tyrosinase) gene fragments. Numbers on nodes are posterior probabilities / bootstrap support values. Asterisks (*) indicate 1.0 posterior probability / 100% bootstrap support. The tree shows the phylogenetic placement of three putatively new species previously recognized for the genus (Santos et al. 2020b), and the dashed rectangle indicates the new species being described here.

FIG. 2.—*Crossodactylodes serranegra*, holotype in life (CFBH 44887, male, SVL 17.1 mm). (A) Dorsal and (B) ventral views, showing nocturnal coloration; (C) diurnal coloration. A color version of this figure is available online.

FIG. 3.—*Crossodactylodes serranegra*, holotype (CFBH 44887). (A) Dorsal view, (B) ventral view; scale bar = 5 mm. (C) Head in dorsal view and (D) in lateral view, (E) left hand and (F) left foot in ventral view; scale bars = 1 mm. A color version of this figure is available online.

FIG. 4.—Live specimens of *Crossodactylodes serranegra* showing variation in dorsal

pattern. (A) MZUSP 159318, adult female; (B) CFBH 46179, adult female; (C) MNRJ 93959, subadult female; (D) CFBH 44890, adult male; (E) MZUSP 159317, adult male; (F) CFBH 46180, subadult male. A color version of this figure is available online.

FIG. 5.—Geographic distribution of *Crossodactylodes serranegra* and congeners from southeastern and northeastern Brazil. Occurrence records for congeneric species were obtained from Santos et al. (2020a). Geographic coordinates for *C. pinto* are approximate due to the unavailability of exact coordinates. Abbreviation for Brazilian states: BA (Bahia), ES (Espírito Santo), MG (Minas Gerais), and RJ (Rio de Janeiro).

FIG. 6.—Habitat of *Crossodactylodes serranegra* in the “campo rupestre”, Parque Estadual da Serra Negra, state of Minas Gerais, at 1,556 m above sea level. (A) A general view of the habitat; the forest patch where the species occurs is indicated by the black arrow. (B) Detail of the vegetation, which is composed by low trees, shrubs, mosses, lichens, and a high density of bromeliads. (C) A flowered *Vriesea schwackeana* bromeliad in which a juvenile female was found. Also note the presence of another species of bromeliad (*Neoregelia* sp.) in the environment (white arrow heads). (D) A single *Vriesea* cf. *schwackeana* bromeliad, where one male, two females, one tadpole, and one egg were found. A color version of this figure is available online.

FIG. 7.—Comparison of secondary sexual characteristics between an adult (CFBH 46183; A–D) and a subadult (CFBH 46180; E–H) male of *Crossodactylodes serranegra*. The forelimb of adult (A) is hypertrophied and shows a higher number of spine-shaped papillae on nuptial pads than in the subadult specimen (E). The buccal floor and the gular region after removal of *mm. submentalis*, *intermandibularis*, *interhyoideus*, and vocal sac of adult specimen (B–C), showing the presence of vocal slits (black arrows), which are absent in the subadult specimen (F–G). The abdominal cavity after removal of organs of digestive system, showing more developed testes (white asterisks) in relation to kidneys (k) in adult (D) than in the subadult (H) specimen. Scale bars = 1 mm. The superficial submandibular musculature of both specimens is shown in Supplemental Fig. S1, available online. A color version of this figure is available online.