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The evolutionary history of an accidental model organism, the leopard gecko *Eublepharis macularius* (Squamata: Eublepharidae)

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A B S T R A C T

The leopard gecko, *Eublepharis macularius*, is a widely used model organism in laboratory and experimental studies. The high phenotypic diversity in the pet trade, the fact that the provenance of different breeding lines is unknown, and that distinct *Eublepharis* species are known to hybridize, implies that the continued use of *E. macularius* as a model requires clarity on the origin of the lineages in the pet trade. We combine multi-locus sequence data and the first range-wide sampling of the genus *Eublepharis* to reconstruct the evolutionary history of the Eublepharidae and *Eublepharis*, with an updated time-tree for the Eublepharidae. Our sampling includes five of the six recognized species and additional nominal taxa of uncertain status comprising 43 samples from 34 localities plus 48 pet-trade samples. The Eublepharidae began diversifying in the Cretaceous. *Eublepharis* split from its sister genera in Africa in the Palaeocene-Eocene, and began diversifying in the Oligocene-Miocene, with late Miocene-Pliocene cladogenesis giving rise to extant species. The current species diversity within this group is moderately underestimated. Our species delimitation suggests 10 species with four potentially unnamed divergent lineages in Iran, India and Pakistan. All 30 individuals of *E. macularius* that we sampled from the pet trade, which include diverse morphotypes, come from a few shallow *E. macularius* clades, confirming that lab and pet trade strains are part of a single taxon. One of the wild-caught haplotypes of *E. macularius*, from near Karachi, Pakistan, is identical to (10) pet-trade samples and all other captive populations are closely related to wild-caught animals from central/southern Pakistan (0.1–0.5 % minimum pairwise uncorrected *ND2* sequence divergence).

1. Introduction

Model organisms are those that are widely used in laboratory and experimental settings, often to answer broad questions in biology (Fields and Johnston, 2005). These species are selected for traits like their ease of captive care and husbandry, their ability to be manipulated experimentally, short generation times, potentially small genomes, for specific properties relevant to the questions being asked, or simply because of historical contingency — they continue to be used in particular fields because of their use by earlier workers (e.g. Fields and Johnston, 2005; Ankeny and Leonelli, 2011). Many lines of model organisms have been bred for numerous generations in the lab, often from an unknown original wild stock, or multiple lines that have been hybridized, e.g. zebrafish (*Danio rerio*) (Wilson et al., 2014). A major appeal of model

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organisms is their ubiquity and the consequent replicability that manipulation of these lines provides — and today in the genomic era, we can uncover the true identity and history of these model lines (Hedges, 2002; Ankeny and Leonelli, 2011).

Among the most common squamate model organisms is the gekkotan lizard *Eublepharis macularius* (Blyth) of the family Eublepharidae. *Eublepharis macularius* has been the subject of numerous studies in all areas of biology, including physiology (Flores et al., 1994; Crews et al., 1998; Starostová et al., 2009); regeneration (McLean and Vickaryous, 2011; Delorme et al., 2012); phenotypic evolution (Kiskowski et al., 2019); temperature-dependent sex determination (TSD) (Pallotta et al., 2017; Viets et al., 1993); behaviour (Sakata et al., 2002; LaDage and Ferkin, 2006); hybridization and ontogeny (Jančúchová-Lásková et al., 2015; Frynta et al., 2018). In addition, there are community resources to facilitate research, such as guidelines for captive care, an embryonic staging table, and annotated genome (Thorogood and Whimster, 1979;

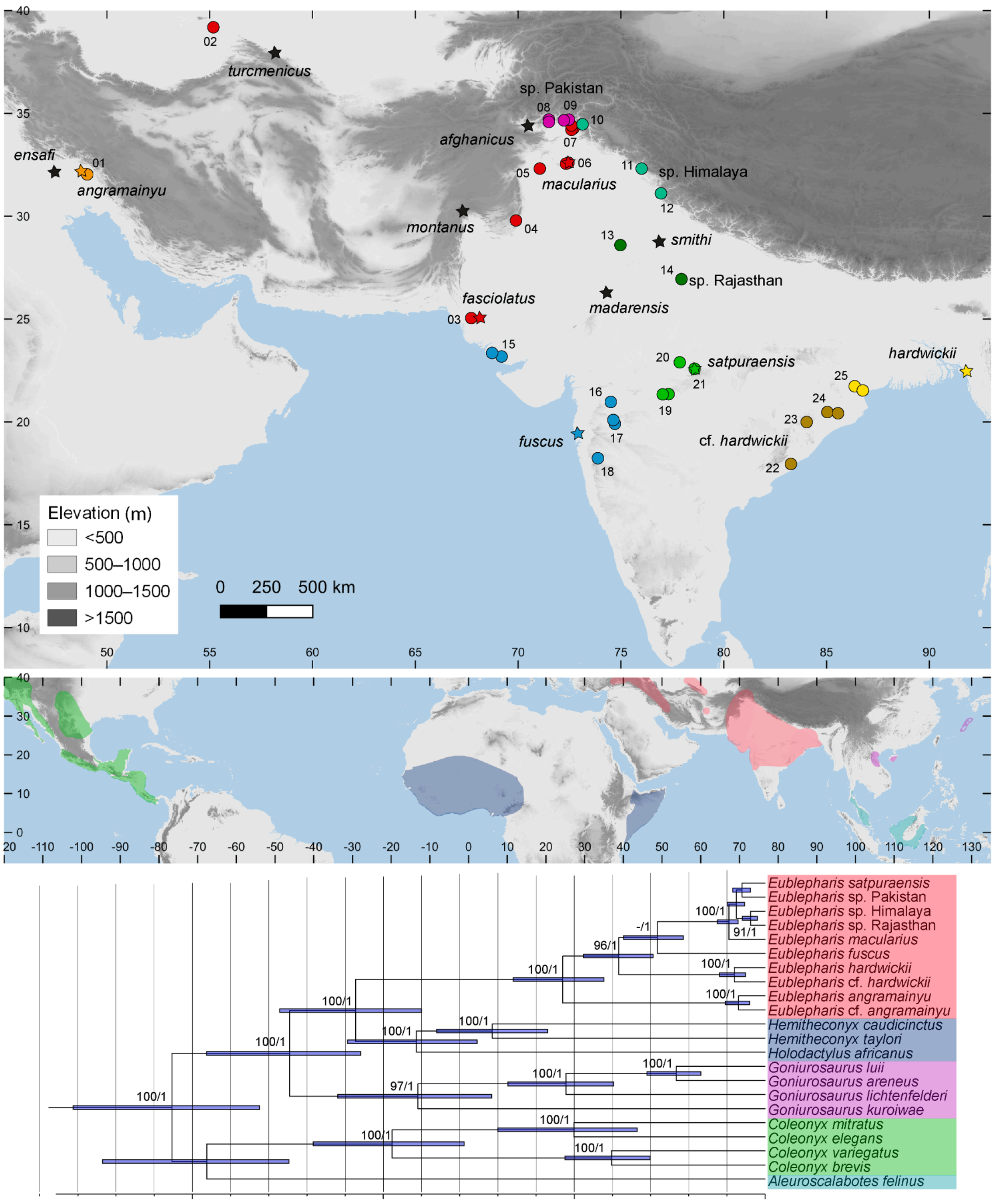

Fig. 1. Type localities and sampling of wild leopard geckos (*Eublepharis*: top panel), distribution of the Eublepharidae (middle panel), and a time-tree for the Eublepharidae (lower panel; based on concatenated nuclear and mitochondrial data, entire time-tree with outgroups shown in Fig. S2). Stars and names in top panel indicate type localities of all available names in the genus, circles and numbers represent sampling locations, and fill colour indicates species (referenced in Table 1, Fig. 2; black fill indicates unsampled taxon). Colours in the middle and lower panels depict the approximate distribution of different genera in the time-tree; node bars represent 95% confidence intervals; bootstrap support/ posterior probability indicated at nodes (only values > 70/ 0.98 shown). Latitude and longitude marked on top and middle panel, axis in lower panel in millions of years ago.

De Vosjoli et al., 2005; Wise et al., 2009; Xiong et al., 2016). In contrast to how well this species is known in captivity in many aspects of its biology, almost nothing is known of its natural history from its native range.

Eublepharis macularius entered the international pet trade at least as far back as the 1960s from various localities in India, Pakistan, and Afghanistan (Mertens, 1959; Minton, 1966; Börner, 1974, 1976, 1981; Werner, 1976; De Vosjoli et al., 2005). Most animals in the pet trade and in established breeding lines are known or believed to have originated from animal dealers in Karachi, Pakistan (Thorogood and Whimster, 1979), but specimens of Indian origin also contributed to commercial breeding stock (De Vosjoli and Tremper, 2005). Through much of the 1990s additional Pakistani animals were legally exported to the European and American pet trade until this was halted in 2000 (Rasheed, 2013). The species was first used as a model organism over 50 years ago (Whimster, 1965), and some captive lines have been bred for over 27 generations (De Vosjoli and Tremper, 2005). *Eublepharis macularius* is also the third most popular reptile pet species (Valdez, 2021). Although most of the pet-trade stock consists of captive-bred geckos, individuals collected from the wild are still occasionally brought into the pet-trade, often illegally (Rasheed, 2013). Studies using *Eublepharis macularius* typically use specimens from the pet trade, but it remains unclear where the source populations of "*E. macularius*" occur, and if these represent pure lines. Additionally, there has been no taxonomic revision of the genus or '*E. macularius*' since Börner (1976, 1981) and Grismer (1988). Furthermore, divergent species within the genus are known to hybridise in captivity (Jančúchová-Lásková et al., 2015), and given how prevalent species complexes are in gekkotans (e.g. Oliver et al., 2010; Grismer et al., 2012; Chaitanya et al., 2019; Agarwal et al., 2021), the continued use of the *E. macularius* model organism requires clarity on the origin of the captive lineages.

Members of the family Eublepharidae are among the most peculiar of the limbed gekkotan families. The only geckos with moveable eyelids, these lizards are unique within the Gekkota for their longevity, with representatives of most genera exceeding 20 years in captivity (Bauer, 2013); their large body size, all genera except *Holodactylus* and most *Coleonyx* attaining snout-to-vent lengths > 100 mm (Feldman et al., 2016); their terrestrial habit and lack of subdigital toe pads (Gamble et al., 2012; excluding the scansorial *Aeluroscalabotes*); and soft, non-calcareous eggs (Werner, 1982; Kluge, 1987). The Eublepharidae is also the least diverse of gekkotan families, with just six genera and 40 described species (Uetz et al., 2021), and most genera are distributed in the Northern Hemisphere at tropical to temperate latitudes with a remarkable intercontinental disjunct distribution (Fig. 1). The six genera include *Aeluroscalabotes* (only one currently recognised species, though more are known (Chang, 2012)) and *Goniurosaurus* (24 species) in East and Southeast Asia; *Coleonyx* (eight species) in the southwestern United States, Mexico and Central America; *Hemitheconyx* and *Holodactylus* (two species each) in east and west Africa; and *Eublepharis* (six species), which is the South and West Asian representative of the Eublepharidae (Uetz et al., 2021; Zhu et al., 2021).

Relationships within the Eublepharidae have been reconstructed using morphological data (Grismer, 1988), mitochondrial sequence data (Ota et al., 1999; Kratochvíl and Frynta, 2002; Jonniaux and Kumazawa, 2008) as well as nuclear data (Gamble et al., 2011, 2012, 2015; Pyron et al., 2013). The most recent common ancestor (mrca) of the Eublepharidae is hypothesized to have a Cretaceous to Jurassic age (Grismer, 1988, Jonniaux and Kumazawa, 2008; Gamble et al., 2011, 2012, 2015). The genus *Eublepharis* is the sister taxon to the African eublepharid genera (Grismer, 1988; Jonniaux and Kumazawa, 2008), and the six recognised species are distributed from eastern India, north and west as far as Turkey (Mirza et al., 2014; Üzüm et al., 2006). *Eublepharis angraimanyu* Anderson and Leviton, 1966 is distributed in Iran, Iraq, Syria and south-eastern Turkey (Al-Sheikhly et al., 2020), *E. turcmenicus* Darevsky, 1977 in Turkmenistan, *E. macularius* in north-west India, Pakistan and Nepal (Rawat et al. 2019), and the remaining species endemic to India – *E. fuscus* Börner, 1974 in western India, *E. hardwickii* Gray, 1827 in eastern India, and *E. satpuraensis* Mirza, Sanap, Raju, Gawai and Ghadekar, 2014 from Central India. *Eublepharis macularius*, the type species of the genus, is a catch-all species with a long taxonomic history including six names that are considered either subspecies or synonyms— *E. afghanicus* Börner from eastern Afghanistan, *E. fasciolatus* Günther from coastal Pakistan, *E. gracilis* Börner from an unknown locality in Afghanistan, *E. madarensis* (Sharma) from northwestern India, *E. montanus* Börner from an imprecise locality along the Pakistan-Afghanistan border, and *E. smithi* Börner from north India (Fig. 1) (Smith, 1935; Das, 1992; Grismer, 1988; Mirza et al. 2014; Uetz et al. 2021).

There has been almost no molecular sampling of *Eublepharis* from its native range or even within the pet trade; just two 'species' have been sequenced from Pakistan and Turkmenistan, and none of the endemic Indian species have previously been sampled. *Eublepharis* species are patchily distributed, and while they may be locally abundant, are generally uncommon and encountered largely during night surveys. Fieldwork on poorly accessible Indian and Pakistani dry zone lizards over the last decade and contributions from colleagues have sampled five of the six recognized *Eublepharis* species and some synonyms of *E. macularius* from the wild, including many from their type localities. Here we aim to (1) provide an updated time-tree for the Eublepharidae based on mitochondrial and nuclear sequence data, (2) reconstruct the evolutionary history of the Eublepharidae and *Eublepharis*, (3) evaluate species diversity within *Eublepharis*, and (4) evaluate the status of the model organism '*Eublepharis macularius*' in the wild and pet trade.

2. . Methods

2.1. Sampling

Eublepharis were opportunistically sampled by AK, DJ, IA, PPM, RM and VG during fieldwork from 2009 to 2020, targeting type localities of known species and synonyms as well as additional localities, with additional contributions of wild-caught specimens and exemplars of *Eublepharis* species and morphs of *E. macularius* from the pet trade including some of known provenance (see acknowledgements). We used the given identities, where available, for samples from the pet trade (Table 1). We generated sequence data for 43 wild-caught samples from 34 localities and an additional 48 pet-trade samples and assigned existing species names to the divergent lineages in our phylogeny based on geographic provenance, with samples from or close to the type localities of all six recognised species (Fig. 1, Table 1; but see sections 3.2, 4.1 for notes on the published sequence of *E. turcmenicus*). DNA was extracted from tail-tips/liver/blood stored in 95–100% ethanol using Qiagen DNeasy extraction kits. We generated up to 2,477 nucleotides (nt) of aligned sequence data including partial sequences for one mitochondrial gene (*ND2*, 1041 nt) and two nuclear genes (*RAG1*, 1041 nt; *PDC*, 395 nt) using published primers and protocols (Table 2). Purification and sequencing of PCR products was outsourced to the Sequencing Facility at the National Centre for Biological Sciences (Bangalore, India), GeneWiz (Plainfield, NJ, USA) and Macrogen Europe (Amsterdam, The Netherlands). Labwork for Indian samples was performed by IA and AM in India, Pakistani samples by DJ in Slovakia, and pet trade samples by IA and TG in the USA. We sequenced complementary strands for increased accuracy (for most pet trade samples). Sequencing using the Macey et al., (1997) primers (in the Indian lab) yielded inadvertent amplification and sequencing of apparent nuclear copies of *ND2* for all *Eublepharis fuscus* from Maharashtra. These sequences had numerous stop codons and a BLAST search (http:// blast.ncbi.nlm.nih.gov/) did not have any similar sequences (<70 % match). These sequences were omitted and we subsequently used the Jonniaux and Kumazawa (2008) primers to amplify *ND2* + genes encoding tRNAs. Sequencing of *ND2* in India used MetF1 only and generated up to ~ 510 nt of sequence data. Due to failure of reverse sequencing reactions and

Table 1
Eublepharis samples used in this study with tissue sample number, locality and Genbank accession numbers. Collection abbreviations: BNHS, Bombay Natural History Society, Mumbai; CAS, California Academy of Sciences, San Francisco; DJ, Daniel Jablonski field series JS, John Scarborough private collection; IAG, Ishan Agarwal field series; MVZ, Museum of Vertebrate Zoology, San Francisco; PMNH, Pakistan Museum of Natural History, Karachi; PPM, Pratyush P Mahapatra field series; ROM, Royal Ontario Museum, Ontario; TG, Tony Gamble field series.

Specimen no	Species	Pet-trade name/ morph	Locality	*ND2*	*RAG1*	*PDC*	Captive/ Wild	Tree and map number
ROM 46,748	*Eublepharis angramainyu*		Iran, Khuzestan	OK563653	OK576482	OK563633	W	01
JS EA1524	*Eublepharis angramainyu*	*angramainyu*	Iran, Khuzestan	OK563654			C	
JS EA1562	*Eublepharis angramainyu*	*angramainyu*	Iran, Ilam	OK563655			C	
JS EA1581	*Eublepharis angramainyu*	*angramainyu*	Iran, Masjed Soleyman	OK563656			C	
JS EA26	*Eublepharis angramainyu*	*angramainyu*	Iran, Kermanshah	OK563657	OK576483		C	
JS EAI13	*Eublepharis angramainyu*	*angramainyu*	Iran, Ilam	OK563658	OK576484		C	
JS EAI14	*Eublepharis angramainyu*	*angramainyu*	Iran, Ilam	OK563659			C	
JS Line2	*Eublepharis angramainyu*	*angramainyu*	Iran, Kermanshah	OK563660	OK576485		C	
TG02278	*Eublepharis angramainyu*	*angramainyu*	Iran, Khuzestan Province	OK563661	OK576486		C	
TG02279	*Eublepharis angramainyu*	*angramainyu*	Iran, Khuzestan Province	OK563662			C	
JS EAM51	*Eublepharis* cf. *angramainyu*	*angramainyu*	Iran, Masjed Soleyman	OK563663			C	
IAG 016 (BNHS 1995)	*Eublepharis fuscus*		India, Maharashtra, Pune			OK563634	W	18
IAG 017 (BNHS 2214)	*Eublepharis fuscus*		India, Gujarat, Kutch	OK563664	OK576487	OK563635	W	15
IAG 053	*Eublepharis fuscus*		India, Maharashtra, Aurangabad	OK563665	OK576488	OK563636	W	17
IAG 054	*Eublepharis fuscus*		India, Maharashtra, Aurangabad	–	OK576489	OK563637	W	17
IAG 178	*Eublepharis fuscus*		India, Maharashtra, Dhule	OK563666	OK576490	OK563638	W	16
IAG 179	*Eublepharis fuscus*		India, Gujarat, Kutch	OK563667	OK576491	OK563639	W	15
IAG 180	*Eublepharis fuscus*		India, Maharashtra, Pune	OK563668	OK576492	OK563640	W	18
IAG 187	*Eublepharis fuscus*		India, Maharashtra, Dhule	OK563669			W	16
IAG 188	*Eublepharis fuscus*		India, Maharashtra, Aurangabad	OK563670			W	17
n/a	*Eublepharis fuscus*	*fuscus*	India	OK563671	OK576493		C	
IAG 189	*Eublepharis hardwickii*		India, Odisha, Balasore	OK563672		OK563641	W	25
IAG 193	*Eublepharis hardwickii*		India, Odisha, Balasore	OK563672	OK576494	OK563642	W	25
PPM 1447	*Eublepharis hardwickii*		India, Odisha, Balasore	OK563673			W	25
IAG 190	*Eublepharis* cf. *hardwickii*		India, Odisha, Kandhamal	OK563674	OK576495	OK563643	W	23
IAG 191	*Eublepharis* cf. *hardwickii*		India, Odisha, Kapilash	OK563675	OK576496	OK563644	W	24
IAG 192	*Eublepharis* cf. *hardwickii*		India, Odisha, Kandhamal	OK563674	OK576497	OK563645	W	23
IAG 196	*Eublepharis* cf. *hardwickii*		India, Andhra Pradesh, Visakhapatnam	OK563676			W	22
PPM 1434	*Eublepharis* cf. *hardwickii*		India, Odisha, Angul	OK563677			W	24
TG02270	*Eublepharis* cf. *hardwickii*	*hardwickii*	female	OK563678			C	
DJ 10,101	*Eublepharis macularius*		Pakistan, Khyber Pakhtunkhwa, Buner	OK563679			W	07
DJ 10,233	*Eublepharis macularius*		Pakistan, Khyber Pakhtunkhwa, Buner	OK563680			W	07
DJ 10,234	*Eublepharis macularius*		Pakistan, Khyber Pakhtunkhwa, Buner	OK563681			W	07
DJ 10,390	*Eublepharis macularius*		Pakistan, Khyber Pakhtunkhwa, Dera Ismail Khan	OK563682			W	05
DJ 7922	*Eublepharis macularius*		Pakistan, Punjab, Dera Ghazi Khan	OK563683			W	04
DJ 7923	*Eublepharis macularius*		Pakistan, Punjab, Dera Ghazi Khan	OK563684			W	04
MVZ 248,432	*Eublepharis macularius*		Pakistan, Sindh, Dadu	OK563685		OK563646	W	03
MVZ 248,433	*Eublepharis macularius*		Pakistan, Sindh, Dadu	OK563686	OK576498		W	03
PMNH 2386	*Eublepharis macularius*		Pakistan, Punjab, Salt Range	OK563687			W	06
PMNH 2387	*Eublepharis macularius*		Pakistan, Punjab, Salt Range	OK563687			W	06
CAS 184,771	*Eublepharis macularius*	*turcmenicus*	Turkmenistan, Krasnovodsk Region, vic. Danata	AF114248,	AY662622	–	C?	02
JS ACHIM	*Eublepharis macularius*	*turcmenicus*	AchimJungfer	OK563688			C	
JS2	*Eublepharis macularius*				EF534776	EF534816	C	
Kumazawa1	*Eublepharis macularius*	*macularius*	n/a	AB308467			C	
n/a	*Eublepharis macularius*	*macularius* brown	n/a	OK563689	OK576499		C	
NC	*Eublepharis macularius*	n/a	n/a	NC033383			C	
TG00081	*Eublepharis macularius*	n/a	Pakistan	JX041350			C	
TG02271	*Eublepharis macularius*	*turcmenicus*	German line	OK563690	OK576500		C	
TG02272	*Eublepharis macularius*	*macularius montanus*	female, Gabor bloodline, Hungary	OK563691	OK576501		C	
TG02273	*Eublepharis macularius*	*macularius montanus*	male, Gabor bloodline, Hungary	OK563691	OK576502		C	
TG02274	*Eublepharis macularius*	*macularius montanus*	female, Gergo bloodline, Hungary	OK563691			C	
TG02275	*Eublepharis macularius*			OK563691	OK576503		C	

(*continued on next page*)

Table 1 (*continued*)

Specimen no	Species	Pet-trade name/ morph	Locality	*ND2*	*RAG1*	*PDC*	Captive/ Wild	Tree and map number
		macularius montanus	female, Gergo bloodline, Hungary					
TG02276	*Eublepharis macularius*	*macularius montanus*	male, Gergo bloodline, Hungary	OK563691			C	
TG02277	*Eublepharis macularius*	*macularius montanus*	female	OK563692			C	
TG02280	*Eublepharis macularius*	n/a	female	AB738955	OK576504		C	
TG02281	*Eublepharis macularius*	n/a	male, breeder box 83	OK563693			C	
TG02282	*Eublepharis macularius*	*macularius fasciolatus*	male	OK563694			C	
TG02283	*Eublepharis macularius*	*macularius fasciolatus*	male	OK563694	OK576505		C	
TG02286	*Eublepharis macularius*	*macularius montanus*	male, Germany bloodline, Breeder box 407	OK563695	OK576506		C	
TG02287	*Eublepharis macularius*	*macularius montanus*	female, Germany bloodline	OK563695			C	
TG02288	*Eublepharis macularius*	*macularius montanus*	male, super giant albino, Godzilla's son, breeder 186	OK563695			C	
TG02289	*Eublepharis macularius*	super giant	male, super giant, Godzilla's grandson	AB738955	OK576507		C	
TG02290	*Eublepharis macularius*		female	OK563696			C	
TG02294	*Eublepharis macularius*	hypo tangerine	female, hypo tangerine	AB738955			C	
TG02295	*Eublepharis macularius*		no data	AB738955			C	
TG02298	*Eublepharis macularius*	*turcmenicus*	male, original from Germany, live	OK563690	OK576508		C	
TG02299	*Eublepharis macularius*	*turcmenicus*	German line	OK563690	OK576509		C	
TG2103	*Eublepharis macularius*		EMAC3?	AB738955			C	
USline	*Eublepharis macularius*	*turcmenicus*	USline	OK563697	OK576510		C	
TG02291	*Eublepharis macularius F* × *Eublepharis angramainyu M*	hybrid	female	AB738955	OK576511		C	
TG02292	*Eublepharis macularius F* × *Eublepharis angramainyu M*	hybrid	female	AB738955	OK576512		C	
TG02293	*Eublepharis macularius F* × *Eublepharis angramainyu M*	hybrid	female, hypo tangerine	AB738955			C	
IAG 015 (BNHS 1980)	*Eublepharis satpuraensis*		India, Maharashtra, Chikhaldhara	OK563698	OK576513	OK563647	W	19
IAG 055	*Eublepharis satpuraensis*		India, Maharashtra, Chikhaldhara	OK563699	OK576514	OK563648	W	19
IAG 172	*Eublepharis satpuraensis*		India, Madhya Pradesh, Nr. Ashapuri	OK563700	OK576515	OK563649	W	20
IAG 181	*Eublepharis satpuraensis*		India, Madhya Pradesh, Pachmarhi	OK563701	OK576516	OK563650	W	21
DJ 9427	*Eublepharis* sp. Himalaya		Pakistan, Khyber Pakhtunkhwa, Battagram	OK563702			W	10
IAG 006	*Eublepharis* sp. Himalaya		India, Himachal Pradesh, Kandaghat	OK563703	OK576517	OK563651	W	12
IAG 010	*Eublepharis* sp. Himalaya		India, Himachal Pradesh, Lunj	OK563704	OK576518	OK563652	W	11
JS KG8	*Eublepharis* sp. Himalaya	*afghanicus*	German line	OK563705	OK576519		C	
TG02284	*Eublepharis* sp. Himalaya	*afghanicus*	male	OK563706	OK576520		C	
TG02285	*Eublepharis* sp. Himalaya	*afghanicus*	female, Germany bloodline	OK563706	OK576521		C	
DJ 10,317	*Eublepharis* sp. Pakistan		Pakistan, Khyber Pakhtunkhwa, Bajaur	OK563707			W	08
DJ 10,318	*Eublepharis* sp. Pakistan		Pakistan, Khyber Pakhtunkhwa, Bajaur	OK563708			W	08
DJ 9455	*Eublepharis* sp. Pakistan		Pakistan, Khyber Pakhtunkhwa, Swat	OK563709			W	09
DJ 9456	*Eublepharis* sp. Pakistan		Pakistan, Khyber Pakhtunkhwa, Swat	OK563710			W	09
DJ 9460	*Eublepharis* sp. Pakistan		Pakistan, Khyber Pakhtunkhwa, Lower Dir	OK563711			W	09
BNHS xx	*Eublepharis* sp. Rajasthan		India, Rajasthan, near Dholpur	OK563712			W	14
n/a	*Eublepharis* sp. Rajasthan		India, Rajasthan, ~25 km NW Pilani	OK563713			W	13
TG00180	*Hemitheconyx caudicinctus*	n/a	n/a	JX041370	HQ426294	HQ426294	C	
CAS 198845	*Holodactylus africanus*	n/a	Kajiado District, Kenya	JX041372	HQ426296	HQ426207	W	

constraints on the export of material from India, we were unable to generate complete *ND2* sequences for those samples.

2.2. Phylogenetic analyses

Sequences were aligned using CLUSTAL W (Thompson et al., 1994) in MEGA 5.2 (Tamura et al., 2011), with translation to amino acids used to verify that the desired protein-coding genes were correctly sequenced. To test the monophyly of the Eublepharidae we used a subset of *Eublepharis* sequences and representatives of all Gekkotan families (see 2.4 *Divergence dating*). Individual gene trees were built for *Eublepharis* using *Hemitheconyx caudicinctus* + *Holodactylus africanus* as

Table 2
Gene, PCR primers, and source. * indicates a sequencing primer; annealing temperatures for all genes was 50–55° C.

Gene	Primer	Source
ND2	MetF1	Macey et al., 1997
	H5934	Macey et al., 1997
	rMet-3L	Jonniaux and Kumazawa, 2008
	GEC ND2	Jonniaux and Kumazawa, 2008
	H5540	Macey et al., 1997
PDC	PHOF1	Bauer et al., 2007
	PHOR2	Bauer et al., 2007
RAG1	RAG1skinkF2	Portik et al., 2010
	RAG1skinkR1200	Portik et al., 2010
	R13	Groth and Barrowclough, 1999
	R18	Groth and Barrowclough, 1999
	RAG1F700*	Bauer et al., 2007
	RAG1R700*	Bauer et al., 2007

outgroups. We used PartitionFinder 2.1 (Lanfear et al., 2016) with the greedy algorithm (Lanfear et al., 2012) and AICc criteria to select the best partitioning scheme and model of sequence alignment for each gene (Table 3). Maximum Likelihood (ML) trees were built using RAxML HPC 8.2.12 (Stamatakis, 2006) implemented on the CIPRES Science Gateway (http://www.phylo.org/; Miller et al., 2010) with ten runs on distinct starting trees, the rapid hill-climbing algorithm and support assessed using 1000 bootstraps. MrBayes 3.2.7 (Ronquist and Huelsenbeck, 2003) was used for Bayesian Inference (BI) on CIPRES using the models and partitions specified in PartitionFinder with model parameters unlinked across partitions and two parallel runs with four chains each (one cold and three hot) run for two million generations sampled every 200 generations; convergence was assessed based on the standard deviation of split frequencies (<0.01) and ESS scores (>200) in Tracer v1.7.1 (Rambaut et al., 2018). Both runs were combined, and a Maximum Clade Credibility tree was built using TreeAnnotator 1.10.4 (Drummond and Rambaut, 2007) with the first 25% of trees discarded as burn-in. Uncorrected pairwise sequence divergence (p-distance) was calculated from the ND2 sequence data in MEGA 5.2.2 using the pairwise deletion option (Table 4).

2.3. Species delimitation

Species delimitation within *Eublepharis* was performed on the *ND2* ML tree with the outgroups dropped, using three variations of tree-based delimitation methods: PTP, bPTP and mPTP (Zhang et al., 2013; Kapli et al., 2017); with bPTP analyses implemented on the web server (http://species.h-its.org/ptp/) using ML for 500,000 generations with a burn-in of 25%, thinning set to 100; and PTP and mPTP analyses run on the web server (https://mptp.h-its.org/) with default settings. We also considered two genetic-distance thresholds: 5% uncorrected *ND2* sequence divergence as indicative of putative species-level divergence (as has been used for geckos, e.g. Grismer et al., 2013; Agarwal et al., 2019); and the lowest genetic divergence between currently recognised species (Table 4).

Table 3
Best fit partitioning scheme and models of sequence evolution for all analyses; cp = codon position.

Data	Partitions	Bayesian Models	ML model
ND2	*ND2* cp1; *ND2* cp2; *ND2* cp3,	TIM + I + G, HKY + G, GTR + G	GTR + G
PDC	*PDC* cp1; *PDC* cp2; *PDC* cp3	K80 + I, F81, HKY	GTR + G
RAG1	*RAG1* cp1, cp2; *RAG1* cp3	HKY	GTR + G
BEAST (*ND2* + Nuclear)	*ND2* cp1; *ND2* cp2; *ND2* cp3, *RAG1* cp1 + *PDC* cp1; *RAG1* cp2; *PDC* cp2; *RAG1* cp3; *PDC* cp3	1,2,5,8: GTR + I + G + X; 3, 4, 6,7: GTR + G + X	GTR + G

2.4. Divergence dating

The dataset for divergence dating used a single lineage per putative *Eublepharis* species from the best species-delimitation solution (see 3.2 Eublepharis *species diversity*) and included additional published eublepharid sequences and representatives of all gekkotan families for all three genetic markers used (Table S1). We estimated divergence dates using BEAST v1.10.4 (Suchard et al., 2018) from the concatenated dataset, using a Yule speciation tree prior, the model of sequence alignment selected in PartitionFinder2 (Table 3) and a lognormal relaxed clock for each partition. We used three fossil calibrations previously proposed within the Gekkota, with exponential priors with a mean of 5 following Agarwal et al. (2020): root Gekkota (offset = 99), the mrca of *Pygopus* and *Paradelma* (offset = 23) and the mrca New Zealand Diplodactylidae (offset = 19). Analyses were run for 100 million generations sampling every 10,000 generations; Tracer v1.7.1 (Rambaut et al., 2018) was used to assess convergence (ESS≫200), and a maximum clade credibility tree was summarized using TreeAnnotator 1.10.4 (Drummond and Rambaut, 2007) with a burn-in of 25%. Dates are reported as median (95% HPD) millions of years ago (mya) in the text. An ML tree (not shown) was also reconstructed using the same dataset to give a measure of bootstrap support for higher-order Eublepharid relationships, using the partitions and model of sequence evolution specified by PartitionFinder 2 and the same RAxML settings as the individual gene trees (see 2.2 *Phylogenetic analyses*).

3. Results

3.1. Phylogeny of the Eublepharidae

The monophyly of the Eublepharidae is well supported, the entire family forming the sister taxon to the Gekkota minus the Pygopodoidea (Fig. 1, Fig. S2). We recovered the same overall topology for the Eublepharidae in ML and BI analyses on the concatenated *ND2* + nuclear data as some previous studies that used molecular sequence data (Gamble et al., 2011, 2012, 2015; Pyron et al., 2013) except with regard to the placement of *Aeluroscalabotes* (Fig. 1). A basal split within the Eublepharidae separates one clade grouping *Aeluroscalabotes* + *Coleonyx* from another one grouping *Eublepharis, Goniurosaurus, Hemitheconyx* and *Holodactylus*. Within the latter clade, *Eublepharis* is the sister taxon to *Hemitheconyx* + *Holodactylus*, and these three genera collectively form the sister taxon to *Goniurosaurus*. All genera and nodes above the genus level receive high support (BS ≥ 99, PP 1.0), except for the sister-taxon relationship of *Aeluroscalabotes* and *Coleonyx*. This is also the major discrepancy between our phylogeny and others that place *Aeluroscalabotes* as the sister to taxon to other eublepharids (e.g. Grismer, 1988; Kratochvíl and Frynta, 2002; Jonniaux and Kumazawa, 2008).

3.2. Eublepharis species diversity

Eublepharis is well-supported as monophyletic in all individual gene trees (except in ML analyses with *PDC*) (Fig. 2, Fig. S1). The *ND2* tree shows *E. angramainyu* as the sister taxon to a clade comprising all other *Eublepharis*. Within the latter clade, *E. hardwickii* is the sister taxon to a clade of the remaining species, and *E. fuscus* is the sister taxon to an *E. macularius* clade comprising *E. macularius*, *E. satpuraensis*, and three unnamed species lineages (Fig. 2). The nuclear data had few informative characters and the same four clades are retrieved with little to no structure within them, though the *E. macularius* clade is collapsed and some samples from the *E. macularius* clade in the *ND2* tree fall outside it (Fig. S1). Within the *E. macularius* clade *ND2* sequence divergence varies from 4.1 to 8.1 % between five broad lineages: *E. macularius*, *E. satpuraensis*, *Eublepharis* sp. Himalaya, *Eublepharis* sp. Pakistan and *Eublepharis* sp. Rajasthan. *Eublepharis angramainyu* and *E. hardwickii*

Table 4
Pair-wise uncorrected genetic distance between putative *Eublepharis* species. Mitochondrial *ND2* (1041 bp), numbers in bold along the diagonal represent the average within group distance (maximum) for putative species with multiple samples.

		1	2	3	4	5	6	7	8	9	10
1	*E. angramainyu*	**1.0 (2.5)**									
2	*E.* cf. *angramainyu*	6.4	–								
3	*E. fuscus*	23.6	24.8	**0.9 (2.2)**							
4	*E. hardwickii*	27.9	27.1	22.3	**0.1 (0.2)**						
5	*E.* cf. *hardwickii*	28.5	27.4	22.8	9.0	**1.3 (3.5)**					
6	*E. macularius*	23.4	23.4	17.0	22.9	23.0	**1.0 (2.9)**				
7	*E. satpuraensis*	21.9	22.8	17.0	23.0	23.7	7.0	**0.3 (0.6)**			
8	*E.* sp. Rajasthan	21.7	24.0	16.3	22.7	22.6	6.1	8.1	**0.6**		
9	*E.* sp. Himalaya	23.3	23.5	17.7	24.6	24.8	6.3	7.9	4.1	**1 (2.5)**	
10	*E.* sp. Pakistan	23.8	23.0	17.4	22.1	21.8	7.6	7.2	7.0	7.6	**2.1 (3.6)**

each include two deeply divergent lineages (6.4–9.0 % uncorrected *ND2* sequence divergence); *E. fuscus* and *E. macularius* have two and three shallowly divergent lineages, respectively (<2.9 % uncorrected *ND2* sequence divergence). The known localities within each of the three shallow lineages of *Eublepharis macularius* include: A) topotypical samples and Dera Ismail Khan, Pakistan; B) type locality of *E. fasciolatus* and Dera Ghazi Khan, Pakistan; C) Buner, Pakistan (Fig. 2). *Eublepharis macularius* from the type locality and from the type locality of *E. fasciolatus* show 1.3 % divergence. The only available sequence of *E. turcmenicus*, supposedly from close to the type locality, is 0.5–0.8 % divergent from other members of the *E. macularius* subclade, suggesting the sequence may be misidentified and is likely a pet-trade *E. macularius* (see Discussion 4.1).

Species delimitation analyses using mPTP recognised 10 species, and PTP and bPTP converged on 11 species (using a threshold support of 0.5), with *Eublepharis* sp. Himalaya and *Eublepharis* sp. Rajasthan additionally split from each other in the latter analyses (Table 5, Table S2). The lowest genetic distance between previously recognised species excluding '*E. turcmenicus*' was 7.0 % (*E. macularius* vs. *E. satpuraensis*); while the lowest interspecific genetic distance in the 10 and 11 species solution was 3.5 % (but up to 5.2 % intraspecific divergence between *Eublepharis* sp. Himalaya and *Eublepharis* sp. Rajasthan in the 10 species solution). The 5% threshold joins *E.* cf. *hardwickii* from Vizag with the rest of *E.* cf. *hardwickii* from the 11-species solution, while applying the lowest genetic distance (7.0 %) between recognized species as a threshold additionally merges *E. angramainyu* and *E.* cf. *angramainyu*, and *E. macularius*, *Eublepharis* sp. Rajasthan, and *Eublepharis* sp. Himalaya into single species (Table 5). As PTP and bPTP analyses with 11 candidate species recovered one species with low genetic divergence from its sister species (*E.* cf. *hardwickii* Vizag), which is represented by a single sample; and mPTP with the 10-species solution lumped two fairly divergent lineages that occupy very different biogeographic regions (*Eublepharis* sp. Rajasthan and *Eublepharis* sp. Pakistan), we conservatively favor the 5 % genetic divergence threshold, which suggests 10 potential species within our sampling of *Eublepharis* (Fig. 2; Table 5, Table S2).

Pet trade samples identified as '*angramainyu*' and '*fuscus*' group with their respective species, and '*hardwickii*' groups with *E.* cf. *hardwickii* in both mitochondrial and nuclear trees. The two known hybrids that we sampled for nuclear data (TG02291–02292; *Eublepharis macularius* F × *E. angramainyu* M) group with *E. macularius* in the mitochondrial tree (Fig. 2) and with *E. angramainyu* in the *RAG1* tree (Fig. S1). Within the *macularius* group in the *ND2* tree, purported samples of '*afghanicus*' group with *Eublepharis* sp. Himalaya, all '*turcmenicus*' group with *macularius* from its type locality (except the previously published sequence of a specimen purported to be from close to the type locality (CAS 184771), which is in the *fasciolatus* subclade), as do the only two '*macularius fasciolatus*' and one '*macularius*', and all other samples fall in the *fasciolatus* subclade including '*macularius*', '*macularius montanus*', and a 'brown morph' of *E. macularius*. One wild haplotype of *Eublepharis macularius*, from near Karachi, Pakistan, is identical to 10 pet-trade samples and differs by a single base from seven others; another from the type locality differs by a single base from four pet-trade samples; and all other captive populations are closely related to wild-caught animals from central/southern Pakistan (0.2–0.5 % minimum pairwise uncorrected *ND2* sequence divergence from wild-caught samples).

3.3. Divergence dating

The final BEAST analysis converged after 100,000,000 generations (ESS values > 200 for all parameters after burn-in). Our divergence estimates for the mrca of the Eublepharidae 78 (91–66 million years ago, mya) overlap broadly with those of Gamble et al. (2015) and are considerably more recent than Jonniaux and Kumazawa (2008). The split between *Aeluroscalabotes* and *Coleonyx* was at about 73 (87–62) mya and between *Goniurosaurus* and the mrca of *Eublepharis* + the African genera at 62 (73–53) mya, and 46 (55–38) mya between the African genera. *Eublepharis* diverged from the African genera 54 (64–45) mya, with sequential divergences separating *E. angramainyu* 27 (33–21) mya, then *Eublepharis hardwickii* 19 (24–15) mya, *E. fuscus* 14 (19–11), and then the *E. macularius* group (Fig. 1). Diversification within the *E. macularius* group and the *angramainyu* and *hardwickii* clades was all within the last 3–5 (6–2) mya.

4. 4. Discussion

4.1. Phylogeny and species diversity

This is the first multi-locus phylogeny of *Eublepharis* and confirms the monophyly of the genus (Grismer, 1988; Jonniaux and Kumazawa, 2008). Our conservative estimate of diversity within the genus is 10 species. There are divergent *Eublepharis* lineages in the Eastern Ghats of peninsular India (cf. *hardwickii*), the Western Himalayas of Northwest India and Pakistan (sp. Himalaya and sp. Pakistan), western India (sp. Rajasthan) and at the extreme western limit of the genus (cf. *angramainyu*; Nazarov, 2017). Our sampling of type localities and others nearby demonstrates that *E. fasciolatus* is genetically very similar in mitochondrial sequence data (1.2–1.4% divergence) to *E. macularius* and thus considered here as a junior synonym thereof. The single published sequence of a purportedly topotypical '*Eublepharis turcmenicus*' is within the *fasciolatus* subclade of *E. macularius*, and other samples identified as *E. turcmenicus* from the pet-trade without specific locality information group with topotypic *E. macularius*. It seems likely that the supposed wild-caught sequence was inadvertently swapped with a pet trade sequence, given that *E. turcmenicus* strongly differs in morphology from *E. macularius,* overlapping partially with *E. angramainyu* (Grismer, 1988, 1991); and the type locality of *turcmenicus* is a considerable distance (>1,800 km) from sampled *E. macularius*.

Some species within this large-bodied, terrestrial, long-lived (at least 37 years; Berghof, 2019), and low-diversity group show little or no differentiation in the nuclear data and low levels of mitochondrial sequence divergence across hundreds of kilometres. Mitochondrial

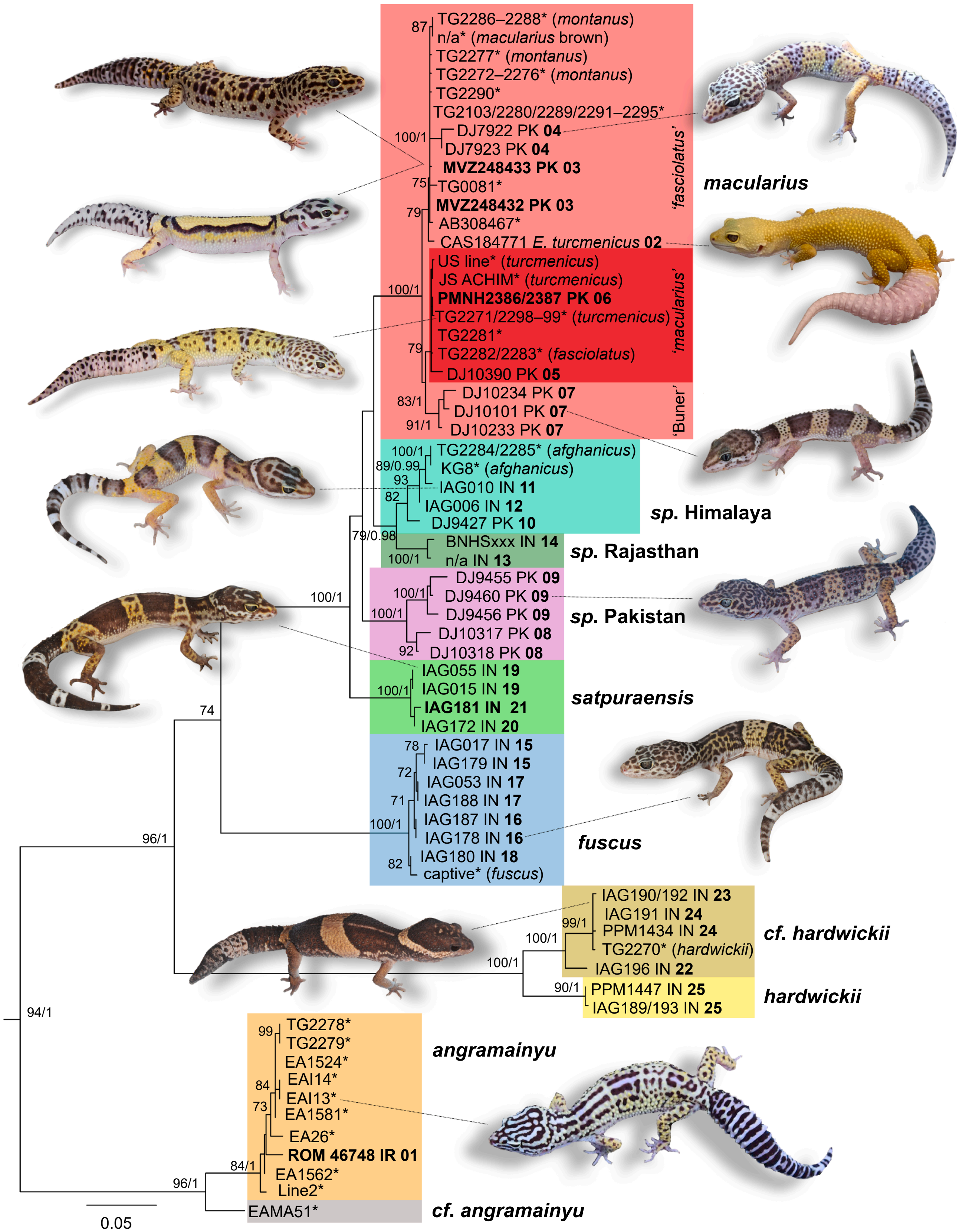

Fig. 2. Maximum likelihood phylogeny of *Eublepharis* based on the *ND2* gene with representative photographs (connected to the sample or clade they represent in the phylogeny by a line); species names in bold; bootstrap support/ posterior probability indicated at nodes (only values > 70/ 0.98 shown); outgroups not shown (see Fig. S2 for complete tree). Country code shown for wild-caught samples (IN = India, IR = Iran, PK = Pakistan); * indicates pet-trade sample (morph/ trade name if known shown in parentheses); colours and numbers in bold following the country codes reference Fig. 1 (top panel), Table 1; horizontal text labels within *E. macularius* indicate subclades; samples from type localities in bold (MVZ248432–33 represent the type locality of *E. fasciolatus*). Photographs of *Eublepharis* by IA (Indian samples), DJ (Pakistan samples), TG (*E. macularius* pet-trade morphs) and John Scarborough (*E. angramainyu*).

Table 5
Species delimitation using different criteria (see methods for details). Alternating light and dark grey within each column indicate which species were recognised using different methods.

Species	mPTP	PTP	bPTP	5% *ND2* divergence	Lowest *ND2* divergence (7%)
E. angramainyu					
E. cf. *angramainyu*					
E. fuscus					
E. hardwickii					
E. cf. *hardwickii*					
E. cf. *hardwickii* VIZAG					
E. satpuraensis					
E. macularius					
E. sp. Rajasthan					
E. sp. Himalaya					
E. sp. Pakistan					

sequence divergence in the widely distributed species (*Eublepharis fuscus*, *E. macularius*, and *E.* sp. Himalaya) is just 2.2–2.9% across distances of ~ 600–1200 km between the farthest localities). In contrast, *Eublepharis* cf. *hardwickii* and *E.* sp. Pakistan have mitochondrial sequence divergence of 3.5–3.6% within ~ 100–300 km. Additionally, there is overlooked diversity across multiple biogeographic regions (Figs. 1, 2). Deeply divergent *Eublepharis* species have been known to hybridise in captivity with viable F1 offspring (Jančúchová-Lásková et al., 2015), and a genome-scale dataset is essential to understand species limits and gene flow within the divergent mitochondrial lineages. Additional geographic sampling throughout the range of the genus and especially in western parts of Afghanistan, Iran, Pakistan and Turkmenistan is vital to track the boundaries of species lineages.

4.1.1. *Eublepharis taxonomy*

Apart from the original descriptions of *Eublepharis*, which date back to 44–194 years ago (except for *E. satpuraensis*; Mirza et al., 2014), and the character-based taxonomic review of Grismer (1988), there has been little taxonomic work on the group, and the genus is generally poorly represented in collections (e.g. only 371 records on VertNet; http://www.vertnet.org/). Additionally, *Eublepharis* species show ontogenetic variation in colour and pattern, making the use of colouration in taxonomic diagnoses problematic (e.g. Börner 1974, 1976, 1981; Grismer, 1988; Mirza et al., 2014). There has been controversy over the validity of *Eublepharis* species names proposed by Achim-Rüdiger Börner in papers in the self-published journals *Miscellaneous Articles in Saurology* (1974) and *Saurologica* (1976, 1981). The primary question is whether these journals constitute publications under Article 8 of the *International Code of Zoological Nomenclature* (ICZN, 1999) (Wagner et al., 2016). However, correspondence with Dr. Börner (21 January 2018) has confirmed that original copies of these publications were prepared by offset printing in runs of 100–150 copies and were distributed widely to institutions at the time of publication. Thus, names originating in these publications, *E. gracilis, E. afghanicus, E. montanus* and *E. smithi* are unambiguously available names; the latter three treated by Grismer (1988) as synonyms of *E. macularius* and *E. gracilis* considered a synonym of *E. macularius* by Grismer (1988) and a nomen dubium and a likely senior synonym of *E. afghanicus* by Wagner (2016). Our sampling did not include *E. gracilis*; nor any topotypical samples of *E. afghanicus*, *E. madarensis, E. montanus*, or *E. smithi*, besides the published *E. turcmenicus* sequence that is identical to pet trade *E. macularius*. *Eublepharis* sp. Himalaya and *Eublepharis* sp. Pakistan show some characters that match the original description of *E. afghanicus* and others that are not consistent (DJ unpubl. data); and *Eublepharis* sp. Rajasthan may represent *E. madarensis* or *E. smithi* – two species of unknown status, with our sampled localities approximately halfway between their type localities (~170–250 km). *Eublepharis madarensis* was described as a 'luminous' (in error) species of *Cyrtodactylus* (Sharma, 1980), and is currently considered a synonym of *E. macularius* (Das, 1992). *Eublepharis ensafi* was described from close to the type locality of *E. angramainyu* (Baloutch and Thireau, 1986) and was synonymized by Grismer (1989) based on a comparison of the type series of *E. angramainyu* with the description of *E. ensafi*.

4.2. *Eublepharis biogeography*

The family Eublepharidae originated in the Cretaceous (Grismer, 1988; Jonniaux and Kumazawa, 2008). The disjunct distribution of extant genera, and the poor support for the grouping of *Aeluroscalabotes* + *Coleonyx*, which differs from previously published phylogenies that place *Aeluroscalabotes* as the sister taxon to other eublepharids (Grismer, 1988; Kratochvíl and Frynta, 2002; Jonniaux and Kumazawa, 2008), precludes rigorous ancestral area reconstructions. We consider the most likely scenario an Asian or Laurasian ancestor for the group, with *Coleonyx* dispersing to the New World through the Beringean land bridge (as previously hypothesized by Grismer, 1988; Gamble et al., 2011). It is unclear where the ancestors of the African genera or the African genera + *Eublepharis* were distributed, and reconstructions within *Eublepharis* are equivocal for an Indian or Saharo-Arabian origin of the group (not shown). However, our data are consistent with *Eublepharis* dispersing into India after the Indian plate collided with Eurasia 55–35 mya (Karanth, 2021). The time of divergence of the mrca of *Eublepharis* + African genera was during a period of global warmth, while early diversification within *Eublepharis* overlaps with both a cool and a warm phase in the early and late Oligocene (Zachos et al., 2001). A very long branch separates the mrca of *Eublepharis* from the mrca of *Eublepharis* + the African genera, indicative of extinctions in the early history of the genus. Grismer (1988) considered *Eublepharis hardwickii* the sister taxon to other members of the genus and speculated that this split was caused by Miocene uplift of the Himalayas. However, our data recover *Eublepharis angramainyu* as the sister taxon to a clade containing other members of the genus, and this latter clade clearly has an Indian origin (not shown). The separation of *E. angramainyu* and the ancestor of the remaining species does, however, overlap with major periods of initial Himalayan uplift.

Eublepharis spp. live in dry open habitats; only *E. hardwickii*, *E.* cf. *hardwickii* and *E. satpuraensis* occur in deciduous forests; an apparently derived condition. The Indian plate was ancestrally forested and wet, with Indian dry-zone diversity traditionally thought to be made up of relatively recent intrusive elements (e.g. Mani, 1974). *Eublepharis* adds to the growing list of dry-zone squamate taxa that have an ancient history in India (e.g. Agarwal et al., 2014; Agarwal and Karanth, 2015;

Agarwal and Ramakrishnan, 2017; Deepak et al., 2018; Lajmi et al., 2020), which suggests that the Indian dry zone dates back to at least the Oligocene (as also suggested by a phylogeny of teresomatan caecilians, Gower et al., 2016). Other dry-zone Indian gekkotans (=gekkonids) that overlap in distribution with *Eublepharis* species and diversified in the same time frame as Indian *Eublepharis* include the rupicolous '*Cyrtopodion' aravallensis* complex and the terrestrial *Hemidactylus gracilis* clade (Agarwal et al., 2014; Lajmi et al., 2020).

The only other lizard genus with a similar distribution in the Indian and Saharo-Arabian regions is *Ophisops*, which has a Saharo-Arabian origin and came into India 30 (34–26) mya with a second Saharo-Arabian subclade dispersing out of India 19 (23–14) mya (Agarwal and Ramakrishnan, 2017). The basal split between *E. angramainyu* and other *Eublepharis* overlaps temporally with the dispersal of *Ophisops* into India from Saharo-Arabia, suggesting that arid-adapted groups were able to disperse between the regions during the late Oligocene.

The highest diversity within our sampling of *Eublepharis* is in northern Pakistan, where representatives of three clades of the *Eublepharis macularius* group are found at similar altitudes within just 80 km of each other (Fig. 1, Fig. S3). These three clades are non-sister and diverged from each other in the late Miocene to Pliocene, 5–4 (6–3) mya, a time of intensified aridification (Zachos et al., 2001) during which the Himalayas may have already been close to modern elevations (e.g. Gébelin et al., 2013; Deng and Ding 2015). This small area in the Khyber Pakhtunkhwa Province of Pakistan is where the Indus River Basin, the outer Himalayas (Siwaliks) and the lower Hindukush mountains (Kabul River Valley) meet; as well as where the Oriental and Palearctic realms transition (Sindaco and Jeremčenko, 2008). Each of the three clades appears to correspond to one of these geographic features — *E. macularius* (Indus Basin), *Eublepharis* sp. Himalaya (Siwaliks) and *Eublepharis* sp. Pakistan (Kabul Valley), suggesting the meeting of these clades reflects the complex geography of the region. The Indus River tracks the division between the Palearctic and Oriental realms and appears to be the main barrier between *E.* sp. Himalaya and the other two species; while a mountain peak and the Swat River are possible barriers between *E. macularius* and *E.* sp. Pakistan. Much more sampling is needed in these topographically diverse regions to uncover patterns of diversity, distribution and potential hybridization events within *Eublepharis*.

4.3. The source of the model organism *Eublepharis macularius*

Like other model organisms, *Eublepharis macularius* is extremely well-known in the lab, and yet we know almost nothing about them in the field. Our sampling of *E. macularius* from Pakistan indicates that they are not exceptionally genetically diverse (≤2.9% mitochondrial sequence divergence) across a distributional range that spans > 1,100 km straight line distance between our most widely spaced samples (assuming that the published *E. turcmenicus* sequence is in error; see 4.1.1 *Phylogeny, Species Diversity and Taxonomic Implications*) and elevations ranging between ~ 120–1800 m. All 30 leopard geckos in the pet trade that we sampled, which include a diversity of morphotypes, come from two shallow clades within *E. macularius*. As the pet trade has been the source of the laboratory populations of *E. macularius*, its continued use as a model organism appears warranted since the animals being used all represent lineages from within a single species, as against being a complex of species. Ten pet-trade animals that we sampled are identical to and seven differ by a single base from a wild-caught haplotype from near Karachi, Pakistan corroborating Thorogood and Whimster (1979), and four others differ by a single base from the topotypic haplotype. This clearly indicates Karachi and somewhere in the vicinity of the Salt Range are two sources for captive material, and since all captive populations are closely related (0.2–0.5 % minimum pairwise uncorrected *ND2* sequence divergence) to wild-caught animals from central/southern Pakistan.

CRediT authorship contribution statement

Ishan Agarwal: Conceptualization, Formal analysis, Data curation, Investigation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration. **Aaron M. Bauer:** Writing – original draft, Writing – review & editing, Resources, Funding acquisition. **Tony Gamble:** Conceptualization, Investigation, Writing – review & editing, Resources, Funding acquisition. **Varad B. Giri:** Investigation, Writing – review & editing. **Daniel Jablonski:** Investigation, Writing – review & editing, Visualization, Resources, Funding acquisition. **Akshay Khandekar:** Investigation, Writing – review & editing. **Pratyush P. Mohapatra:** Investigation, Writing – review & editing. **Rafaqat Masroor:** Investigation, Writing – review & editing. **Anurag Mishra:** Investigation, Writing – review & editing. **Uma Ramakrishnan:** Writing – review & editing, Resources, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2022.107414.

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