Phylogeny and classification of fejervaryan frogs (Anura: Dicroglossidae)

Eugenia Sanchez1, S. D. Biju2, Mohammed M. Islam3, Mahmudul Hasan3, Annemarie Ohler4, Miguel Vences1 & Atsushi Kurabayashi1,6

1 Zoological Institute, Technische Universität Braunschweig, Mendelssonstr. 4, 38106 Braunschweig, Germany
2 Systematics Lab, Department of Environmental Studies, University of Delhi, Delhi 110007, India
3 Amphibian Research Center, Hiroshima University, Higashi-Hiroshima, Japan
4 Department of Fisheries Biology & Genetics, Bangamata Sheikh Fazilatunnesa Mujib Science & Technology University, Melandah, Malancha-2012, Jamalpur, Bangladesh
5 Institut de Systématique, Évolution, Biodiversité, ISYEB – UMR 7205 – CNRS, MNHN, UPMC, EPHE, Muséum national d’Histoire naturelle, Sorbonne Universités, 57 rue Cuvier, CP 30, 75005, Paris, France
6 Unit for Environmental Sciences and Management, North-West University, Potchefstroom, 2520, South Africa

Corresponding author: Miguel Vences, e-mail: m.vences@tu-braunschweig.de

Abstract. Systematics and classification of Asian frogs of the genus Fejervarya and related genera (family Dicroglossidae; hereafter referred to as fejervaryan frogs) have been the subject of intensive debates in the past few years. We complement previous phylogenetic studies with analyses of concatenated sequences from 14 nuclear loci and mitochondrial gene fragments, totaling 12,752 nucleotides for 46 species representing all major lineages and relevant outgroups. We find strong support for two major clades within Fejervarya: a South Asian clade and a Southeast Asian clade. Previously, South Asian species have been hypothesized to constitute a separate genus, Zakerana (currently considered a junior synonym of Fejervarya), and also include species previously described as members of the genus Minervarya. Although parsimony and species tree analyses found support for the monophyly of Fejervarya as currently understood, partitioned Bayesian inference and unpartitioned Maximum Likelihood analyses of concatenated sequences recovered Southeast Asian species as a clade sister to the genus Sphaerotheca, albeit with low nodal support. We discuss the advantages and disadvantages of alternative classification schemes in light of previously proposed criteria for naming supraspecific taxa. The current single-genus taxonomy would impart desirable economy of nomenclatural change, and morphological diagnosability. However, other taxon naming criteria such as support for monophyly, temporal framework for diversification, and biogeographic regionalism would support a contrasting two-genus alternative. Because new species of Fejervarya are increasingly being discovered and described, and because a single-genus classification (Fejervarya) will remain controversial, given ambiguous support for its inferred monophyly, we propose recognizing two genera: Southeast Asian Fejervarya, and South Asian Minervarya. This classification results in two genera whose monophyly is strongly supported, respectively, and unlikely to be challenged by future analyses. Accordingly, we transfer all species of the South Asian clade to the genus Minervarya.

Key words. Amphibia, Fejervarya, Minervarya, Sphaerotheca, Zakerana, systematics, Taxon Naming Criteria.

Introduction

The Asian frogs variably assigned to the genera Fejervarya Bolkay, 1915, Minervarya Dubois, Ohler & Biju, 2001, and Zakerana Howlader, 2011, popularly referred to as Rice Frogs (e.g., Sumida et al. 2007), Cricket Frogs (e.g., Suwannapoom et al. 2016), or fejervaryan frogs, represent a major unsolved phylogenetic and taxonomic conundrum. Currently, Minervarya and Zakerana are considered junior synonyms of Fejervarya (Dinesh et al. 2015, Frost 2017). Fejervarya frogs, members of the family Dicroglossidae Anderson, 1871, are the closest relatives to the genus Sphaerotheca Günther, 1859. How exactly the different clades of fejervaryan frogs are related to each other, and to Sphaerotheca, is however disputed. Phenotypically, most taxa are inconspicuous, and many are difficult to distinguish by morphology, yet are genetically and ethologically diverse, as is evident from a recently marked increase of new species descriptions (e.g., Kuramoto et al. 2007, Howlader et al. 2016, Garg & Biju 2017). Molecu-
lar phylogenetic analyses have included a variable number of species, and recovered either the genera Fejervarya and Sphaerotheca as sister groups (Pyron & Wiens 2011, Dinesh et al. 2015), or recovered Sphaerotheca nested within Fejervarya (Frost et al. 2006, Kotaki et al. 2008, 2010, Hasan et al. 2014).

In general, previous studies all found support for two monophyletic groups within Fejervarya (Dinesh et al. 2015), one consisting of a clade primarily limited to South Asia, and another largely occurring in East and Southeast Asia (e.g., Kurabayashi et al. 2005, Kotaki et al. 2008, Dinesh et al. 2015). Of these, the Southeast Asian clade, which includes the type species of Fejervarya (Rana limnomaris Gravenhorst, 1829), has most often been found as sister to Sphaerotheca (Frost et al. 2006, Kotaki et al. 2008, 2010, Hasan et al. 2014). In contrast, the South Asian clade has been hypothesized to constitute a distinct genus Zakerana, based on peripheral molecular and morphological comparisons (Howlader 2011) which however were based on incomplete taxon sampling. Further complicating fejervaryan taxonomy, an additional genus has been previously proposed to accommodate a group of small-bodied South Asian species (Minervarya Dubois, Ohler & Biju 2001), which have later been found nested within the larger South Asian clade (Kuramoto et al. 2007, Dinesh et al. 2015).

In this work, we reconsider the systematics of fejervaryan frogs with the dual goals of (1) reconstructing phylogeny with an expanded dataset consisting of both nuclear and mitochondrial DNA sequences, and (2) evaluating implications of alternative generic taxonomy, following explicit taxon naming criteria (Vences et al. 2013).

Methods

Sequences of species from the genera Fejervarya, Sphaerotheca, Euphlyctis, Nannophrys, Hoplobatrachus and Limnonectes (family Dicroglossidae) were retrieved from GenBank for the nuclear and mitochondrial gene fragments: 12S ribosomal RNA (12S), 16S ribosomal RNA (16S), chemokine (C-X-C motif) receptor 4 (cxcr4), cytochrome b (cob), sodium/calcium exchanger 1 (ncx1), recombination activating gene 1 (rag-1; included as two different fragments named rag-1-3' and rag-1-5'), recombination activating gene 2 (rag-2), rhodopsin exon 1 (rho), tyrosinase (tyr), and brain-derived neurotrophic factor (bdnf). For some taxa, we further complemented the dataset for these genes with new sequences generated via standard polymerase chain reactions (PCR; Table S1). Additionally, we used the same DNA extractions as Kotaki et al. (2010) to sequence fragments of leucine-rich repeat / WD repeat-containing protein (kiaa1239), sascin (sacs), and titin (ttn) with primers and nested PCR as in Shen et al. (2012), and pro-opiomelanocortin (pomp) using standard PCR. Primers and PCR conditions are provided in Table S1. Chromatograms were visually checked and edited using CodonCode Aligner v3.0.3 (CodonCode Corporation, Centerville, MA, USA). Newly generated sequences were submitted to GenBank (accession numbers MG719866-MG719921; Table SM2). Institutional abbreviations used herein are RBRL (Rondanlo Biodiversity Research Laboratory, St. Aloysius College, Mangalore, India) and ZSI (Zoological Survey of India); for additional institutional abbreviations, see Table SM2. Sequences were aligned using ClustalW (Thompson et al. 1994) as implemented in MEGA 5.2 (Tamura et al. 2011). Alignments of mitochondrial gene fragments (12S and 16S) were processed with Gblocks 0.91b (Castresana 2000) to remove ambiguously aligned sections, with 50% threshold and default settings. Flanking positions of the selected blocks are provided in Table SM3. Best-fitting models of molecular evolution and partition schemes were inferred by the Akaike Information Criterion (AIC) using PartitionFinder 2 (Lanfear et al. 2016) and are provided in Table SM4. Bayesian inference (BI) phylogenetic analyses of the concatenated DNA sequences were performed with MrBayes 3.2 (Ronquist et al. 2012). Our analysis was run for 20 million generations, with four Markov Chains (three heated and one cold), and trees were sampled every 1,000 generations. We confirmed stabilization and convergence of likelihood values using Tracer 1.4 (Ramaut & Drummond 2007). After discarding 25% of sampled trees as burn-in, a majority-rule consensus tree was used to summarize relationships, with posterior probabilities of nodes used to assess support. Several sequences from multiple species of Limnonectes were combined chimerically and designated as a distant outgroup; and sequences of Euphlyctis, Nannophrys and Hoplobatrachus were included as hierarchical outgroups. We also reconstructed topologies under the Maximum Parsimony (MP) optimality criterion in PAUP* v4.10 (Swofford 2002) using the tree-bisection-reconnection (TBR) branch-swapping algorithm and 100 random addition sequence replicates, and conducted bootstrap analyses of 2,000 pseudoreplicates with 10 random addition sequence replicates each. Furthermore, we estimated phylogeny under Maximum Likelihood (ML), implementing a GTR+G substitution model. From the ML analysis we obtained a best tree and a majority consensus rule tree of 1,000 full bootstrap replicates in RAxML V 7.2.7 (Stamatakis 2006). We also inferred a species tree using “BEAST” v.1.8.4 (Drummond et al. 2012), defining all nuclear genes as separate unlinked partitions, and merging all mitochondrial genes into a further partition, with a Yule prior and a relaxed clock model, and implementing GTR+G substitution models for each partition. The analysis was run for 500 million generations, and a consensus tree was generated after discarding 50% of sampled trees as burn-in using TreeAnnotator v.1.8.4 (Drummond et al. 2012).

To explore individual gene histories, we estimated separate gene trees with BI and ML for each locus. A final BI analysis was performed on a smaller matrix of the concatenated dataset, in which all nucleotide positions with missing data were excluded, and at least two representative species from each main clades were included (resulting in the exclusion of the gene pomp from this analysis).
Results

Our preferred phylogenetic inference is based on DNA sequences from 14 genes, four of which were newly sequenced in this study (kiaa1239, pomc, sacs and ttn; Table SM2). Our final matrix contained 46 terminals, and 14.5% of the sequences were newly obtained for this study. After alignment and exclusion of hypervariable sites with Gblocks, the matrix consisted of 12,752 nucleotide sites for each terminal (having 52.5% missing data; i.e., total 278,631 nucleotides). Of all 12,752 sites, 2,860 were variable and 1,699 were parsimony-informative.

Bayesian Inference analysis of the concatenated dataset produced a largely resolved tree in which fejervaryan frogs (and, thus, the genus *Fejervarya* sensu lato; Frost 2017) are paraphyletic with respect to the genus *Sphaerotheca* (Fig. 1). The two primary clades are recovered with strong support (posterior probabilities PP=1.0). The South Asian clade contains species previously assigned to *Minervarya*, whereas the Southeast Asian clade was recovered sister...
to *Sphaerotheca*, albeit with low support (PP=0.88). Our ML analysis yielded a tree virtually identical to our BI estimate (Fig. SM17), whereas MP provided moderate support (bootstrap proportions, BS=64%) for *Fejervarya* sensu lato (with both South and Southeast Asian clades) as a monophyletic group, together constituting the group sister to *Sphaerotheca* (Fig. SM18). Also, the species tree analysis (with *BEAST*) is different from the concatenated BI and ML analyses in providing strong support (PP=0.98) for monophyly of *Fejervarya* sensu lato (Fig. SM18).

To evaluate whether missing data might have influenced the placement of the two primary clades of *Fejervarya* (relative to *Sphaerotheca*), we analyzed a concatenated matrix reduced to eight taxa and a single outgroup, with only nuclear genes included, and pomc excluded. The topology of the resulting BI tree (Fig. SM1) was virtually identical to our preferred topology (Fig. 1), with an increased support for the placement of *Sphaerotheca* with the Southeast Asian clade (PP=0.9). In contrast, an additional analysis including only mtDNA sequences recovered *Sphaerotheca* closely related to the South Asian clade (PP=0.81; Fig. SM1). Nearly all single-gene trees (Figures SM2–16) recovered both South Asian and Southeast Asian *fejervaryan* clades (usually with weak support) and groupings of either of these clades with *Sphaerotheca*. Only a single individual gene tree (sacs) provided strong support for the monophyly of *Fejervarya* sensu lato relative to *Sphaerotheca* (Table 1).

### Discussion

In this study, we assembled the largest dataset of mitochondrial and nuclear gene sequences compiled to date for the purpose of elucidating evolutionary relationships of *fejervaryan* frogs (*Fejervarya* sensu lato *Frost* 2017) and the related genus *Sphaerotheca*. Our results agree with previous studies by recovering three main subclades, corresponding to (1) genus *Sphaerotheca*, (2) South Asian clade, and (3) Southeast Asian clade (*Frost* et al. 2006, *Kuramoto* et al. 2007, *Kotaki* et al. 2008, 2010, *Howlader* 2011, *Pyron & Wiens* 2011, *Hasan* et al. 2014, *Dinesh* et al. 2015). Furthermore, our results unambiguously find support for a relationship in which taxa previously referred to *Minervarya* are nested within the South Asian clade (*Kuramoto* et al. 2007, *Hasan* et al. 2014, *Dinesh* et al. 2015). However, despite the inclusion of over 10 kbp of sequence data for 14 loci, relationships among the three subclades could not be satisfactorily resolved.

Although we anticipate that more extensive phylogenomic datasets may provide, in the future, robust estimates of topology and resolution of this persistent phylogenetic conundrum, we can also imagine that relationships and classifications proposed to accommodate them may remain controversial. At present, both Bayesian and likelihood analyses of concatenated nuclear and mitochondrial genes suggest the non-monophyly of *fejervaryan* frogs, whereas only the species tree found strong support for *fejervarya* monophyly, and parsimony found moderate support for it. Consequently, the clade stability for *Fejervarya* sensu lato is weak, i.e., a clade including South Asian and South East Asian species and excluding species of *Sphaerotheca* is not consistently recovered by varying methods of analysis and datasets (*Vences* et al. 2013), necessitating a discussion of alternative classification strategies.

We consider the following: (1) should *fejervaryan* frog species all be contained within a single, maximally inclusive genus (*Fejervarya*) or (2) separated into two or more smaller, regionalized genera? Finally, (3) if splitting is
Classification of fejervaryan frogs

shown to be the superior solution, which higher-level genus names should be applied to the monophyletic entities discovered here?

How many genera of fejervaryan frogs?

Clearly, reaching a consensus on the generic classification of *Fejervarya* could generate disagreement and debate, requiring a justified resolution of what could become a complex taxonomic issue; we acknowledge that any solution will necessarily remain subjective. In the following, we contribute to this discussion by evaluating the alternatives following the Taxon Naming Criteria (TNCs) of Vences et al. (2013). Of the originally conceived TNCs, three have been highlighted as particularly important (Vences et al. 2013): monophyly, robustness of clades to varying analysis methods and datasets (“clade stability”), and phenotypic diagnosability.

According to the data presented here, monophyly of fejervaryan frogs (South Asian taxa + Southeast Asian taxa), to the exclusion of *Sphaerotheca*, can neither be ascertained by concatenated multigene BI and ML analyses, single-gene trees, nor by separate concatenated analyses of mitochondrial and nuclear DNA sequences. However, the conceivable monophyly of a clade in which these two subclades are sister groups also cannot be refuted. In fact, some previous studies have supported such a monophyletic arrangement (Pyron & Wiens 2011) with strong nodal support, as did the MP analysis of our extensive dataset (Fig. SM18) and the species tree analysis (Fig. SM20). The Monophyly TNC, therefore, does not necessarily result in a need to change the existing classification — because in terms of a general economy of taxonomic change, unstable, back-and-forth changes in classification should be avoided (Vences et al. 2013). However, recent phylogenomic studies have shown that some controversial nodes of the Tree of Life are recalcitrant to unambiguous resolution even when millions of nucleotide positions are analyzed (e.g., Rodríguez et al. 2017). It is conceivable that the node that joins the two main clades of fejervaryan frogs, even if real, may never be unambiguously resolved and strongly supported in the foreseeable future.

The Phenotypic Diagnosability TNC also argues for the recognition of a single, more inclusive genus to accommodate all fejervaryan frogs. The species are morphologically very similar, and identification of a phenotypic distinction between South Asian versus Southeast Asian species has not been forthcoming (Ohler et al. 2014). One recognizable diagnostic character, a putative synapomorphy, shared by all fejervaryan species is their conspicuous ventrolateral lines (Fig. 2), whereas these structures are absent in other amphibians including *Sphaerotheca* (Dubois et al. 2001, Garg & Biju 2017).

On the contrary, classification of fejervaryan frogs into two genera, corresponding to South Asian and Southeast Asian regional clades, would be favored by the Clade Stability TNC of Vences et al. (2013). Each of the two clades received strong heuristic support and both were supported in nearly all single-gene analyses. If these three clades (when also considering *Sphaerotheca*) each are considered distinct genera, we would consider it unlikely that the monophyly of individual genera could be challenged in the near future.

Three further secondary TNCs should also be considered at this point, which would be most consistent with a two-genus classification scheme: first, the Time Banding (time calibration comparisons) TNC follows suggestions of Avise & Mitchell (2007), in which clades of similar evolutionary age might best be recognized at equivalent taxonomic “ranks”. Our phylogeny generally demonstrates similar genetic scales of divergences among the branches separating the three clades; although we did not attempt an explicit dating analysis, this crude generalization suggests at least a generally comparable scale of temporal diversification among the main clades. Thus, we favor an interpretation resulting in the assignment of all three clades to equivalent rank, namely separate genera. Second, the Biogeography TNC favors classifications that maximize the biogeographic information. This criterion logically is consistent with a two-genus arrangement, which would facilitate discussion and analysis of distribution patterns of the primary respective clades. One of these contains a majority of South Asian (Indian subcontinent) taxa, but also a species from the Andaman Islands (F. cf. andamanensis), and some taxa from Thailand (e.g., an undescribed taxon previously named F. sp. hp3; Kotaki et al. 2010). In contrast, the Southeast Asian clade contains species primarily from Southeast Asia, but also extends to Bangladesh (F. cf.

Figure 2. Ventral view of *Fejervarya limnocharis* from Bogor, Java, Indonesia, showing the presence of “fejervaryan lines,” shared by members of *Fejervarya* and *Minervarya* (absent in *Sphaerotheca*).
moodiei) and India (*F. orissaensis*). Finally, third, although based on isolated evidence only, the Hybrid Viability TNC may also support classifying our primary clades as separate genera, as crossing experiments have revealed complete hybrid incompatibility between some species (Sumida et al. 2007, Islam et al. 2008). For example, Southeast Asian *Fejervarya* sp. “Large” produced viable hybrids when crossed with the equally Southeast Asian *F. iskandari*, whereas hybrids resulting from crosses with a representative of the South Asian clade, *Fejervarya* sp. “Medium”, were not viable, i.e. complete hybrid incompatibility at embryonic stage (Islam et al. 2008).

**Morphological variation and the phylogenetic position of Minervarya**

Many species of fejervaryan frogs are morphologically similar; we suspect that many more morphologically cryptic (but genetically distinct) new species will be discovered and described in the future. However, despite this prevailing pattern, our phylogeny clarifies ranges of phenotypic variation in the clades defined here, and aids in the identification of morphologically distinct subgroups.

For instance, the phylogenetic position of *Minervarya* (as defined by Ohler et al. 2014), at first glance, is at odds with the definition of *Fejervarya* sensu lato. Its placement within the South Asian clade is confirmed by our multigene analyses (Fig. 1 and Fig. SM17–20) and by several single-gene trees (Table 1). In particular, molecular data are available for *M. sahyadris*, the type species of *Minervarya*, from Aralam, India (voucher RBRL 050714-01; Hasan et al. 2014), as well as for a specimen from the type series of *M. gomantaki* (voucher ZSI/WGRC/V/A/867 = CESF 2294; Dinesh et al. 2019), a phenotypically typical *Minervarya* species (Ohler et al. 2014). These small frogs are nestled within the South Asian clade but are morphologically distinct from other dicログロスノリス igfrods by their (1) smaller body, (2) white horizontal stripe on the upper lip, and (3) presence of a rictal gland (Dubois et al. 2001, Ohler et al. 2014). Substantial morphological variation in the South Asian clade also is conspicuously evident in five species of the *Fejervarya rufescens* subclade (*F. cephi*, *F. kadar*, *F. manoharani*, *F. neilcoxi*, and *F. rufescens*; Garg & Biju 2017). These species notably resemble members of the genus *Sphaerotheca*, but differ by the presence of the fejervaryan lines and other morphological characters such as the presence of an outer metatarsal tubercle and smooth ventral body skin (Garg & Biju 2017).

**Proposal for a classification of fejervaryan frogs in two genera**

In this study, we compiled a large molecular phylogenetic dataset, yet were unable to provide unambiguous support for the monophyly of fejervaryan frogs. And although *Fejervarya* was paraphyletic with respect to *Sphaerotheca* in a subset of our individual gene trees, we likewise failed to identify unambiguous heuristic (bootstrap, posterior or probabilities) support for such a relationship from all analyses. The respective nodes are, for the time being, best viewed as characterizing an unresolved polytomy. Identification of an unambiguous morphological synapomorphy (the derived presence of fejervaryan lines) which, apparently, is unique to fejervaryan taxa, serves to aid in the recognition and definition of the clade containing the South Asian + Southeast Asian taxa, as it is absent in *Sphaerotheca*, but might not reliably guide inference of relationships given the overall high amount of homoplasy observed in ranoid anuran morphology. After carefully evaluating relevant TCNs of Vences et al. (2013), we conclude that distinguishing between a single-genus versus two-genera solution for generic boundaries in fejervaryan frogs is hampered by ambiguous alternatives, guided solely by subjective assessment of the merits of TCNs and taxonomic distribution of character states and, thus, requires a somewhat arbitrary resolution.

Given past disagreements concerning the taxonomic arrangement of *Fejervarya*, we prefer an arrangement that treats South Asian and Southeast Asian taxa each as a separate genus. This is because, despite the clear ambiguity and anticipated remaining controversy (see comments by Frost 2017), we feel that a biogeographically sensible, regionally circumscribed arrangement has a higher probability of stabilizing the taxonomy of these frogs. We note that such a more atomixed classification will not likely be challenged in future phylogenetic studies focusing on the question of monophyly versus paraphyly of fejervaryan taxa. We espouse this primary, geographically logical argument, based on the premise that recognition of two regionally defined and biogeographically delimited clades/genera of fejervaryan frogs, will result in stability and diminished controversy—and we feel that this scheme is in better agreement with the Time Banding, Biogeography, and Hybrid Viability TCNs (Vences et al. 2013).

The Southeast Asian species sampled here include *Rana limnocharis* Gravenhorst, 1829 (the type species of *Fejervarya*), which is the first-described genus-group name in the group (Frost 2017). The second clade (primarily South Asian taxa) contains the type species of both *Minervarya* and *Zakerana* (*Minervarya sahyadris* Dubois, Ohler, and Biju, 2001; and *Rana limnocharis* syhadensis Annandale, 1919), of which *Minervarya* was described earlier and, thus, has nomenclatural priority.

Of the 45 described species of *Fejervarya* (Frost 2017), 31 were included in our phylogenetic analysis and can definitively be assigned to either of the two proposed genera based on independent empirical results (genetic data, and species’ positions in our phylogeny). For some species, morphological similarity-based arguments were presented by Ohler et al. (2009), allowing us to assert putative overall phenotypic assignments for selected taxa not sampled in our genetic dataset. We propose the following amended taxonomy, allocating all species of *Fejervarya* sensu lato Frost (2017) to either *Fejervarya* or *Minervarya*; we none-
theless are aware that in some cases, confirmation will be required to confidently place unsampled taxa in one of the two genera. Individual problematic species’ assignments may be challenging. It is also worth emphasizing that the identity of some of the historically described species (e.g., *M. brevipalmata*) will require additional scrutiny, due to apparently lost type specimens and doubtful type locality (Frost 2017). The identity and validity of numerous other taxa likewise require confirmation; for instance, *M. asmati* (Howlader, 2011) may be a junior synonym of *M. pirei* (Dubois, 1975), judging from genetic similarity inferred here (Fig. 1).

**Fejervarya Bolkay, 1915**


**Minervarya Dubois, Ohler & Biju, 2001**

Type species: *Minervarya sahyadris* Dubois, Ohler, and Biju, 2001 by original designation.

Junior synonym: *Zakerana Howlader, 2011*

Type species of *Zakerana: Rana limnocharis syhadrensis* Annandale, 1919 by original designation.


Vences, M., J. M. Guayasamin, A. Miralles & I. de la Riva (2013): To name or not to name: Criteria to promote economy of change in Linnaean classification schemes. – *Zootaxa, 3656*: 201–244.

Supplementary material

Supplementary Tables SM1–SM4.

Supplementary Figures SM1–SM20.