Establishing a national fungal genetic resource to enhance the bioeconomy

T. S. Suryanarayanan, Venkat Gopalan*, Dinkar Sahal and Kaustuv Sanyal*

Global conservation activities of animals and plants to protect endangered species are laudable. Similarly, various national and international bodies have recognized the value of preserving different types of microbes, the 'hidden-constituents' that are present in all habitats. However, conservation of microbial biodiversity has generally not been a priority in the world. We present a roadmap for creating a national genetic resource for fungi, whose diversity reflects their remarkable fitness for the rich and varied habitats and environments in India. In addition to offering fine prospects for research-based higher education, this national asset will accelerate technology development and the bioeconomy.

Keywords: Fungal diversity, culture collections, microbial cultures, conservation.

IN India, sustained efforts by the Government, researchers and the general public have contributed to the success of conservation programmes such as Project Tiger (launched in 1973), mirroring comparable global initiatives aimed at protecting iconic animals (e.g. the giant panda) or plants (e.g. cycads). The conservation of microscopic organisms, from bacteria to algae, fungi and protozoa by maintaining collections of living cultures has similarly been endorsed by various groups as equally valuable, both due to the economic potential[1] and to the critical role of microbes in the success of habitat-preservation programmes. Yet, conservation of microbes, especially fungi, has not been a priority either in India or elsewhere (with some exceptions, see below). Even the Member States of the Rio Convention on Biological Diversity did not emphasize conservation of fungi[2], leading to David Minter's sobriquet for fungi as 'the orphans of Rio'! (DOI: http://dx.doi.org/10.1016/j.cub.2010.09.024).

Fungi cannot be classified as either plant or animal, and are now known to be an independent kingdom of life sharing a common ancestor with animals and not at all closely allied to plants. In fact, their unique structural, growth and reproductive properties, and special biochemical attributes lend appeal to fundamental and applied studies of these diverse organisms. Because of the tremendous variations in their life histories and ecological strategies, Heilmann-Clausen *et al.*[3] have suggested customized, case-specific approaches to achieve success with respect to their conservation. We extend this idea by arguing that India, home to numerous apparently unique fungi in its rich habitats and ecosystems, could focus on conservation and development of fungi that hold potential for the nation's growing bioeconomy.

Why have fungi not been given the importance they deserve?

Arguably, fungi constitute the most species-rich kingdom and are estimated to support at least 1.5 million species and probably 3 or even 5 million, based on recent large-scale metagenomics studies[4,5]. Fungi are ubiquitous and occur in soil, freshwater and sea water, either as symbionts or parasites of plants and animals. Some live in extreme habitats having unusually high levels of salt, acidity, alkalinity, temperature, heavy metals or ionizing radiation. They perform important ecosystem processes, including nutrient recycling by acting on simple and recalcitrant substrates. Despite enormous species and functional diversity, fungi have typically not been considered for conservation efforts due to various reasons[6]. Foremost, their microscopic nature (certainly not as charismatic as a tiger or an elephant for non-mycologists and policy makers) has led to an undervaluation of their roles in maintaining different habitats and in global ecosystem processes such as the carbon cycle. Second, while it is easier to estimate the loss of habitats and extinction of larger species (the major focus of conservation efforts), there are limited data indicating loss of fungal species due to elimination of habitats or hosts. Finally, the general

T. S. Suryanarayanan is in the Vivekananda Institute of Tropical Mycology, Ramakrishna Mission Vidyapith, Chennai 600 004, India; Venkat Gopalan is in the Department of Chemistry and Biochemistry and Center for RNA Biology, The Ohio State University, Columbus, OH 43210, USA; Dinkar Sahal is in the Malaria Research Laboratory, International Centre for Genetic Engineering and Biotechnology, Aruna Asaf Ali Marg, New Delhi 110 067, India; Kaustuv Sanyal is in the Molecular Mycology Laboratory, Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur, Bengaluru 560 064, India.
*For correspondence. (e-mail: t_sury2002@yahoo.com; gopalan.5@osu.edu)

negative perception is that fungi cause either disease or spoilage of food and materials. This is partly due to the fact that even the few papers in conservation-centric journals that deal with the role of fungi focus on their roles as pathogens rather than their vital contributions to new technologies or all four categories of ecosystem service as recognized by the Millennium Ecosystem Assessment[2,3,7] (e.g. aiding the roots of a plant to acquire soil N and P).

Why study fungi?

Microbial resources can be employed to address many of the current global challenges pertaining to health, food, energy and environment. Being one of the most ancient eukaryotes to colonize land[8], fungi have evolved to survive in diverse ecological niches with varying growth conditions. Such remarkable ecological fitness, the basis of their competitive edge, is partly due to phenotypic versatility, which in turn stems from genetic variability attributable to genetically different nuclei in their mycelia[9] and acquisition of novel genes through inter- and intra-kingdom gene transfers[10,11]. Such fungal diversity offers fine prospects for scientific exploration, manpower training and industrial exploitation. In fact, this contention is supported by research on endophytes conducted over the last two decades by the Vivekananda Institute of Tropical Mycology (VINSTROM), a research institute in southern India.

Endophytes infect plants but do not cause any disease in them. VINSTROM has about 1000 endophyte cultures, which were isolated from trees of Mudumalai forest in the Western Ghats, plants of Arunachal Pradesh, mangrove trees of Pichavaram in Tamil Nadu, and marine algae/seagrasses of the coast of Tamil Nadu. VINSTROM's global research collaborations have uncovered the biotechnological potential of some of these cultures. Endophytic fungi elaborate anticancer metabolites and plant-growth regulators[12], antimalarial metabolites[13], antialgal, antifungal, antibacterial and insecticidal metabolites, and novel enzymes for an array of industries[14–19]. If VINSTROM, a small laboratory with minimum resources and a singular focus on one ecological group of fungi (endophytes), could identify several fungal candidates with potentially exploitable traits, a more concerted nationwide effort could be expected to identify unprecedented numbers of fungi with desirable characteristics.

Why another fungal culture collection centre in India?

India has many biodiversity hot spots and supports a remarkable range of ecosystems, including diverse types of forests, grasslands, wetlands, deserts and mangrove ecosystems (coastal and marine) from sea-level to some of the world's highest mountain ranges. India's biodiversity is largely unknown, as exemplified by periodic discoveries even of new plant and animal species[20,21]. Knowledge of the country's biodiversity offers a route to build national bioassets.

About 75% of the industrially used enzymes come from merely five genera of fungi[22], reflecting a phylogenetic bias in those exploited to date. Most of the roughly 120,000 named fungi have never been assessed for potentially exploitable properties; and that 120,000 is at most 7% of all predicted fungi. As the richness of fungal species increases towards the equator[23], the tropics can be expected to harbour most of the predicted but unknown fungal diversity – thus, the diverse ecosystems of India offer an enormous potential for bioprospecting.

It is therefore pertinent to consider the extent to which the mycological infrastructure in India is equipped to support the exploitation of the native fungi of the country[24]. Approximately 27,500 species of fungi are known from India, many of which have not been reported from any other country. This total included 15,500 species on plant litter, 327 on herbivore dung and 450 endophytic fungi[25]. Despite the pioneering efforts of C. V. Subramaninan[26], a mycologist who championed the *ex situ* conservation of fungi in India, a minority, sadly are preserved as living cultures that can be made available for screening for exploitable attributes. The principal collections of living fungi of India are: the National Fungus Culture Collection of India (NFCCI), Pune, with about 2800 strains of diverse groups of fungi (http://nfcci.aripune.org/about_us.php); the Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, has some fungi amongst its over 9000 cultures but focuses on bacteria, yeasts and plasmids (http://mtcc.imtech.res.in/aboutmtcc.php); and the National Bureau of Agriculturally Important Microorganisms (NBAIM) has 700 species belonging to 250 genera of fungi. In contrast, 30,000 fungal cultures are maintained in the UK National Collection of Fungus Cultures[1] and 50,000 by the CBS/KNAW Fungal Biodiversity Centre in the Netherlands (http://www.cbs.knaw.nl/index.php/collection). Developing the living fungal biodiversity maintained in Indian collections towards these levels is a major undertaking, but one which merits serious consideration in view of the long-term benefits that can accrue through innovations in education, research and industry. Such centres are also able to provide training to staff in drug discovery and other bioprospecting companies to empower them to develop their business. As a step towards this goal, we propose building the Fungal Genetic Resource of India (FUNGEN), an initiative whose scope and mission exceeds that of a conventional repository. Unlike the conventional repositories which house taxonomy-based collections of cultures, FUNGEN would focus on a trait (phenotype)-centred collection[27]. Moreover, the development, planning and implementation of FUNGEN are expected to exploit an unusual alliance of multiple stakeholders in our society.

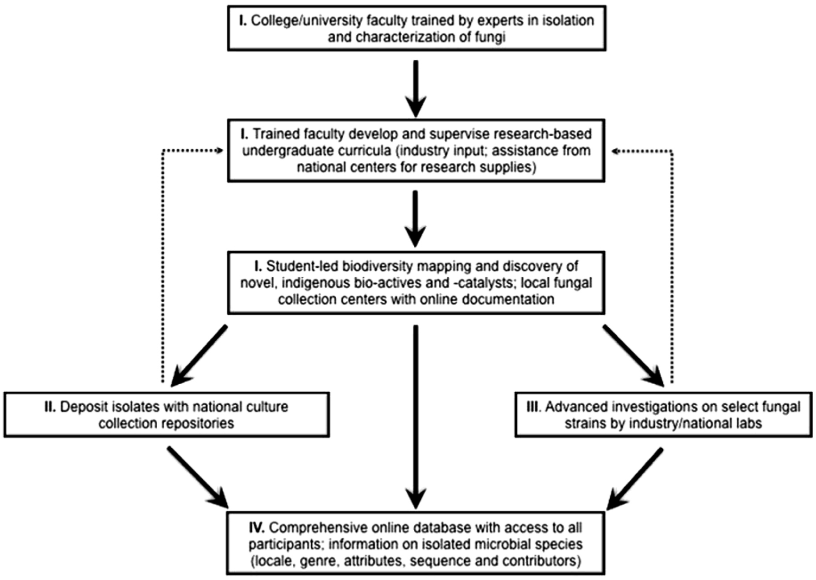

Figure 1. Crowdsourcing model to build a national microbial repository (reproduced with permission from Suryanarayanan and Gopalan[28]).

What is the operational scope of FUNGEN?

Foremost, FUNGEN's mission will adhere to the guidelines set forth by the World Federation of Culture Collections (WFCC), a global monitor of collection standards and practices. The overarching goal of FUNGEN will be to harness the technological potential associated with the fungal biodiversity in the less-studied habitats of India, using a crowdsourcing model that exploits a collaborative network of educational institutions, national research institutes and industries[28] (Figure 1). Skill development and curricular enhancement in different parts of the country should be a high priority of FUNGEN; human resources development in the various processes involved such as isolation, culturing and identification of fungi, molecular techniques and screening organisms for specific bioactivities will add a heuristic approach to higher education.

As to fungal biodiversity, sampling priorities will be guided by Suryanarayanan and Hawksworth[29], who have identified many of the little explored and extreme habitats in which fungi may be found: corals, sponges, deep-sea sediments, resins, gums, soils (alkaline, acidic, hypersaline, contaminated with heavy metals), on and in insects, in the gut and dung of wild animals, plant roots and shoots, lichen, etc. These sources will be studied in collaboration with industrial partners within the framework of research objectives to identify: (i) fungal compounds effective against drug-resistant *Plasmodium falciparum* or *Mycobacterium tuberculosis*, nosocomial infection-causing yeasts, crop pests and pathogens, and (ii) enzymes for clinical therapy and for industry (e.g. plant biomass-deconstruction, bioremediation and enzymes active at high temperatures/salinity and low pH). This list is preliminary and would be developed in consultation according to industrial or societal needs.

How will FUNGEN be operated?

For any collection centre, several operational possibilities exist. Therefore, the following proposal for FUNGEN should only be considered as a starting point for discussions, which we expect will include consultations with directors of NFCCI, MTCC and NBAIM. Clearly, FUNGEN should ideally be physically located in one of the major cities with excellent infrastructure and skilled manpower. The management team could consist of a director with a long-standing record of leadership and scientific accomplishments in mycology, and assisted by two senior personnel with complementary expertise in educational outreach and industrial liaison. Several staff scientists (M S, Ph D level) will be needed to perform distinct functions: isolation of fungi from challenging sources, design of high-throughput screening methods for bioactives and enzymes, maintenance of fungal cultures submitted from different locations and associated informatics (a searchable database of fungi, including name, location, key attributes, registry of personnel involved; fungal genome sequences, etc.), development of teaching kits and training of college/university instructors. Appropriate administrative support will be essential to keep the centre running smoothly.

While FUNGEN will seek to identify strains with valuable properties and work actively with industry and national institutes, it will be greatly aided by a crowdsourcing effort to rapidly build this national fungal repository[28] (Figure 2). The plan envisions the recruitment of B Sc/M Sc students in colleges to participate in this

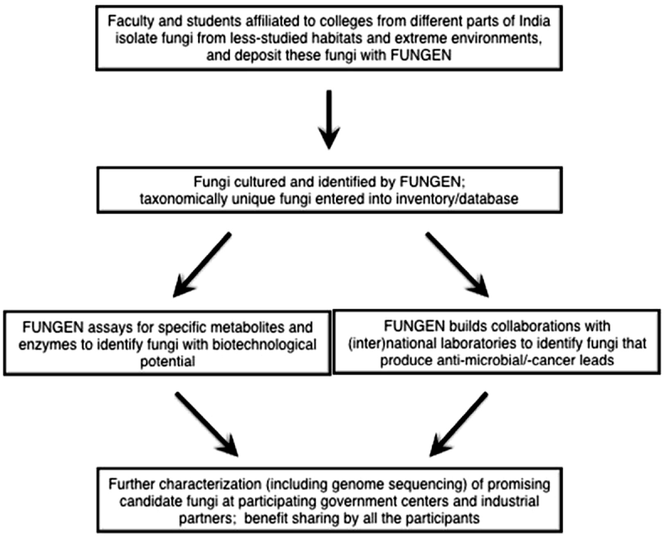

Figure 2. Framework for the overall functioning of FUNGEN.

national initiative while experiencing first-hand the execution of a research project and the excitement of scientific discovery. FUNGEN has two critical roles to play in this regard. First, it will train college faculty in isolation, testing and culturing of fungi from different habitats. For example, students in Andaman Islands, West Bengal, Rajasthan and Kerala could be involved in isolating fungi from the Andaman Sea, Sunderban mangroves, soils of the Thar Desert, and endophytes or litter fungi from near the Silent Valley respectively. Second, although each of the participating colleges can serve as a mini-collection centre's, FUNGEN will obtain each isolate and provide an accession number. FUNGEN will initially parse these collections not by taxonomic identification but by the rapid biodiversity assessment method[30] that uses culture and morphological characteristics. Unique isolates will be maintained for subsequent taxonomic and molecular characterization and inclusion in the central inventory.

Involvement of such multiple mini-centres of collection ensures rapid build-up of cultures and provides solutions to several logistical problems related to transfer of material in a timely fashion. More importantly, it also allows a certain degree of pride and ownership for the 'crowdsourcing' students in each region. Of course, there are challenges imposed by geography and the absolute need for uniformity in training while maintaining rigour in scientific pursuits. FUNGEN could share its training responsibilities with NFCCI and MTCC, with each serving as a regional training centre; if so, the future location of FUNGEN should be chosen so as to triangulate the country suitably. For the research-based curricula to be uniform and consistent, FUNGEN will need to develop a centralized framework for professional development of staff, including trainers and for protocols and reagents to be used for fungal isolation. Participant feedback will allow refinement of initial pilots for subsequent iterations.

How will FUNGEN be financed?

The cost for maintaining a culture collection nominally includes those for staff, acquisition and maintenance of strains, data management, utilities and consumables; however, there is no standard financial model suitable for all culture collection centres[1]. A cost-sharing plan involving State and Central governments is needed. All major culture collections have a substantial amount of on-going governmental support, although some income can be expected from culture sales, training, research grants and industrial collaborations. While teacher salaries, travel costs and infrastructure for the crowd sourcing programme logically remain the fiscal responsibilities of the local colleges and their respective State governments, the Central government would need to bear the costs of FUNGEN's core operation. The corporate social responsibility mandates should be expanded to encompass industrial contributions for the creation of such national repositories and for the training of tomorrow's workforce. Once established and as the holdings grow, FUNGEN will also increasingly generate revenue from the sources noted above.

Summary

The collaboration between a newly created FUNGEN, national laboratories, other culture collection centres, academia and industry has the potential to contribute to overall research and economic development in India. In addition to developing its in-house collections, FUNGEN will also provide a service by integrating a decentralized network of experts, research scholars and existing facilities towards the common objective of fungal collections and usage. An example of what can be discovered by such an enterprise is shown by Liaud *et al.*[31], who screened fungal isolates housed in the International Centre of Microbial Resources in France for the production of organic acids, the basis of a huge industry. Public availability of biomaterials and coordinated efforts between FUNGEN and relevant partners will enhance the value of its collection several fold (see Furman and Stern[32] for other examples), while fully exploiting the biodiversity resources to accelerate India's research and bioeconomy. We urge appropriate departments and agencies of the Government of India to establish a working group of actual and potential stakeholders to take this concept forward and make it a reality.

1. Smith, D., McCluskey, K. and Stackebrandt, E., Investment into the future of microbial resources: culture collection funding models and BRC business plans for biological resource centres. *SpringerPlus*, 2014, **3**, 81–92.
2. Griffith, G. W., Do we need a global strategy for microbial conservation? *Trends Ecol. Evol.*, 2012, **2**, 1–2.
3. Heilmann-Clausen, J. *et al.*, A fungal perspective on conservation biology. *Conserv. Biol.*, 2015, **29**, 61–68.
4. Blackwell, M., The fungi: 1, 2, 3 … 5.1 million species? *Am. J. Bot.*, 2011, **98**, 426–438.
5. Hawksworth, D. L. Global species number of fungi: are tropical studies and molecular approaches contributing to a more robust estimate? *Biodivers. Conserv.*, 2012, **21**, 2425–2433.
6. Heilmann-Clausen, J. *et al.*, Communities of wood-inhabiting bryophytes and fungi on dead beech logs in Europe – reflecting substrate quality or shaped by climate and forest conditions? *J. Biogeogr.*, 2014, **41**, 2269–2282.
7. Pringle, A., Barron, E., Sartor, K. and Wares, J., Fungi and the Anthropocene: biodiversity discovery in an epoch of loss. *Fungal Ecol.*, 2011, **4**, 121–123.
8. Heckman, D. S., Geiser, D. M., Eidell, B. R., Stauffer, R. L., Kardos, N. L. and Hedges, S. B., Molecular evidence for the early colonization of land by fungi and plants. *Science*, 2001, **293**, 1129–1133.
9. Angelard, C., Tanner, C. J., Fontanillas, P., Niculita-Hirzel, H., Masclaux, F. and Sanders, I. R., Rapid genotypic change and plasticity in arbuscular mycorrhizal fungi is caused by a host shift and enhanced by segregation. *Int. Soc. Microb. Ecol. J.*, 2014, **8**, 284–294.
10. Wenzl, P., Wong, L., Kwang-Won, K. and Jefferson, R. A., A functional screen identifies lateral transfer of b-glucuronidase (gus) from bacteria to fungi. *Mol. Biol. Evol.*, 2005, **22**, 308–316.
11. Scazzocchio, C., Fungal biology in the post-genomic era. *Fungal Biol. Biotechnol.*, 2014, **1**, 7.
12. Suryanarayanan, T. S., Thirumalai, E., Prakash, C. P., Govindarajulu, M. B. and Thirunavukkarasu, N., Fungi from two forests of southern India: a comparative study of endophytes, phellophytes and leaf litter fungi. *Can. J. Microbiol.*, 2009, **55**, 419–426.
13. Kaushik, N. K., Murali, T. S., Sahal, D. and Suryanarayanan, T. S., A search for antiplasmodial metabolites among fungal endophytes of terrestrial and marine plants of southern India. *Acta Parasitol.*, 2014, **59**, 745–757.
14. Govinda Rajulu, M. B., Thirunavukkarasu, N., Suryanarayanan, T. S., Ravishankar, J. P., El Gueddari, N. E. and Moerschbacher, B. M., Chitinolytic enzymes from endophytic fungi. *Fungal Divers.*, 2011, **47**, 43–53.
15. Govinda Rajulu, M. B., Lai, L. B., Murali, T. S., Gopalan, V. and Suryanarayanan, T. S., Several fungi from fire-prone forests of southern India can utilize furaldehydes. *Mycol. Prog.*, 2014, **13**, 1049–1056.
16. Nagarajan, A., Thirunavukkarasu, N., Suryanarayanan, T. S. and Gummadi, S. N., Screening and isolation of novel glutaminase free L-asparaginase from fungal endophytes. *Res. J. Microbiol.*, 2014, **9**, 163–176.
17. Suryanarayanan, T. S., Thirunavukkarasu, N., Govindarajulu, M. B. and Gopalan, V., Fungal endophytes: an untapped source of biocatalysts. *Fungal Divers.*, 2012, **54**, 19–30.
18. Nagaraju, D., Govinda Rajulu, M. B., El Gueddari, N. E., Suryanarayanan, T. S. and Moerschbacher, B. M., Identification and characterization of chitinolytic enzymes from endophytic fungi. Sugars in Norwich–Royal Society of Chemistry, Carbohydrate Meeting, London, 2009.
19. Venkatachalam, A., Thirunavukkarasu, N. and Suryanarayanan, T. S., Distribution and diversity of endophytes in seagrasses. *Fungal Ecol.*, 2015, **13**, 60–65.
20. Biju, S. D., Sonali, G., Mahony, S., Wijayathilaka, N., Senevirathne, G. and Meegaskumbura, M., DNA barcoding, phylogeny and systematics of Golden-backed frogs (Hylarana, Ranidae) of the Western Ghats–Sri Lanka biodiversity hotspot, with the description of seven new species. *Contrib. Zool.*, 2014, **83**, 269–333.
21. Kumar, K. M. P., Hareesh, V. S., Adsul, A. V., Vimal, K. P., Balachandran, I. and Yadav, S. R., A new species of *Chlorophytum* (Asparagaceae) from southern Western Ghats of India. *Phytotaxa*, 2014, **188**, 282–286.
22. Østergaard, L. H. and Olsen, H. S., Industrial applications of fungal enzymes. In *The Mycota* (ed. Hofrichter, X. M.), Springer, Berlin, 2010, pp. 269–290.
23. Tedersoo, L., Bahram, M., Põme, S., Kõljalg, U. and Yoron, N. S., Global diversity and geography of soil fungi. *Science*, 2014, **346**, 6213.
24. Manoharachary, C., Sridhar, K., Singh, R., Adholeya, A., Suryanarayanan, T. S., Rawat, S. and Johri, B. N., Fungal biodiversity: distribution, conservation, and prospecting of fungi from India. *Curr. Sci.*, 2005, **89**, 58–71.
25. Bhat, D. J., *Fascinating Microfungi (Hyphomycetes) of Western Ghats – India*, Broadway Book Centre, Panjim, 2010.
26. Subramanian, C. V. Tropical Mycology: future needs and development. *Curr. Sci.,* 1982, **51**, 321–325.
27. Aguilar-Trigueros, C. A. *et al.*, Branching out: towards a trait-based understanding of fungal ecology. *Fungal Biol. Rev.*, 2015; http://dx.doi.org/10.1016/j.fbr.2015.03.001.
28. Suryanarayanan, T. S. and Gopalan, V., Crowdsourcing to create national repositories of microbial genetic resources: fungi as a model. *Curr. Sci.*, 2014, **106**, 1196–1200.
29. Suryanarayanan, T. S. and Hawksworth, D. L., Fungi from little-explored and extreme habitats. In *Bio-diversity of Fungi: Their Role in Human Life* (eds Deshmukh, S. K. and Rai, M. K.), Oxford & IBH Publishing, New Delhi, 2005, pp. 33–48.
30. Hyde, K. D. and Hawksworth, D. L., Measuring and monitoring the biodiversity of microfungi. In *Biodiversity of Tropical Microfungi* (ed. Hyde, K. D.), Hong Kong University Press, Hong Kong, 1997, pp. 11–28.
31. Liaud, N. *et al.*, Exploring fungal biodiversity: organic acid production by 66 strains of filamentous fungi. *Fungal Biol. Biotechnol.*, 2014, **1**, 1–10.
32. Furman, J. L. and Stern, S., Climbing atop shoulders of giants: the impact of institutions on cumulative research. *Am. Econ. Rev.*, 2011, **101**, 1933–1963.

ACKNOWLEDGEMENTS. We thank Prof. David. L. Hawksworth, CBE, former President, International Union of Biological Sciences (IUBS), Dr Balakrishna Pisupati, Former Chairman, Biodiversity Authority of India, Chennai and Prof. R. Uma Shaanker, University of Agricultural Sciences, Bangalore for comments on the original draft.

Received 18 May 2015; accepted 15 June 2015

doi: 10.18520/v109/i6/1033-1037