## MOLECULAR APPROACHES IN NATURAL RESOURCE CONSERVATION AND MANAGEMENT

Recent advances in molecular genetics and genomics have been embraced by many scientists in natural resource conservation. Today, several major conservation and management journals are using the "genetics" editors of this book to deal solely with the influx of manuscripts that employ molecular data. The editors have attempted to synthesize some of the major uses of molecular markers in natural resource management in a book targeted not only at scientists but also at individuals actively making conservation and management decisions. To that end, the text features contributors who are major figures in molecular ecology and evolution – many having published books of their own. The aim is to direct and distill the thoughts of these outstanding scientists by compiling compelling case histories in molecular ecology as they apply to natural resource management.

J. Andrew DeWoody is Professor of Genetics and University Faculty Scholar at Purdue University. He earned his MS in genetics at Texas A&M University and his PhD in zoology from Texas Tech University. His recent research in genetics, evolution, and ecology has been funded by organizations including the National Science Foundation, the U.S. Department of Agriculture (USDA) National Research Initiative, the Great Lakes Fishery Trust, and the National Geographic Society. His research is published in more than thirty-five journals, and he has served as Associate Editor for the *North American Journal of Fisheries Management*, the *Journal of Wildlife Management*, and *Genetica*.

John W. Bickham is Professor in the Department of Forestry and Natural Resources (FNR) and Director of the Center for the Environment at Purdue University. He received his MS in biology from the University of Dayton and his PhD in zoology from Texas Tech University. He was on the faculty of Texas A&M University's Department of Wildlife and Fisheries Sciences for thirty years. He has published more than two hundred articles in scientific journals in evolutionary genetics, including comparative cytogenetics, molecular systematics, molecular ecology, and ecotoxicology.

Charles H. Michler is the Fred M. van Eck Director of the Hardwood Tree Improvement and Regeneration Center at Purdue University and Site Director of the National Science Foundation Industry & University Cooperative Research Program's Center for Advanced Forest Systems. He earned his MS and PhD in horticulture, physiology, and biochemistry from The Ohio State University. He has published more than eighty-five scholarly works and has edited nine books and proceedings. He is Editor of *Plant Breeding Reviews* and Associate Editor of the *Journal of Forest Research*.

Krista M. Nichols is Assistant Professor, Departments of Biological Sciences and Forestry and Natural Resources at Purdue University. She received her MS in fisheries and wildlife from Michigan State University and her PhD in zoology from Washington State University. She was a National Research Council postdoctoral Fellow at the National Marine Fisheries Services, Northwest Fisheries Science Center. Dr. Nichols has published in the fields of ecotoxicology, genetics, and ecology.

Olin E. Rhodes, Jr., is Professor in the FNR and Director of the Interdisciplinary Center for Ecological Sustainability at Purdue University. He received his MS in wildlife biology from Clemson University and his PhD in wildlife ecology from Texas Tech University. He was named a Purdue University Faculty Scholar in 2006. He has published more than 135 scholarly works in ecology and genetics and has recently served as Associate Editor of the *Journal of Wildlife Management*.

Keith E. Woeste is a research molecular geneticist for the USDA Forest Service Northern Research Station Hardwood Tree Improvement and Regeneration Center and Adjunct Assistant Professor at Purdue University's FNR. He received an MDiv in theology from the Jesuit School of Theology at Berkeley, an MS in horticulture from the University of California–Davis, and his PhD in genetics from the University of California–Davis.

# Molecular Approaches in Natural Resource Conservation and Management

Edited by

**J. Andrew DeWoody**
Purdue University

**John W. Bickham**
Purdue University

**Charles H. Michler**
Purdue University

**Krista M. Nichols**
Purdue University

**Olin E. Rhodes, Jr.**
Purdue University

**Keith E. Woeste**
Purdue University

CAMBRIDGE
UNIVERSITY PRESS

CAMBRIDGE UNIVERSITY PRESS
Cambridge, New York, Melbourne, Madrid, Cape Town, Singapore,
São Paulo, Delhi, Dubai, Tokyo, Mexico City

Cambridge University Press
32 Avenue of the Americas, New York, NY 10013-2473, USA

www.cambridge.org
Information on this title: www.cambridge.org/9780521731348



First published 2010

Printed in the United States of America

*A catalog record for this publication is available from the British Library.*

*Library of Congress Cataloging in Publication data*

Molecular approaches in natural resource conservation and management /
edited by J. Andrew DeWoody ... [et al.].
p. cm.
Includes bibliographical references and index.
ISBN 978-0-521-51564-1 (hardback) – ISBN 978-0-521-73134-8 (pbk.)
1. Biodiversity conservation. 2. Genetic resources conservation.
3. Molecular genetics. 4. Conservation of natural resources.
I. DeWoody, J. Andrew, 1969– II. Title.
QH75.M647 2010
333.95′16–dc22 2009050318

ISBN 978-0-521-51564-1 Hardback
ISBN 978-0-521-73134-8 Paperback

# Contents

Color plates follow page 174.

# Contributors

**Gerard J. Allan**
Department of Biological Sciences
Environmental Genetics and Genomics Facility
Northern Arizona University
Flagstaff, AZ

**Giridhar N. R. Athrey**
Department of Biology
University of Louisiana
Lafayette, LA

**John C. Avise**
Ecology and Evolutionary Biology
School of Biological Sciences
University of California
Irvine, CA

**Joseph K. Bailey**
Department of Biological Sciences and the Merriam-Powell Center for Environmental Research
Northern Arizona University
Flagstaff, AZ

**Amy B. Baird**
National Museum of Natural History – Naturalis
Leiden, The Netherlands

**Randy K. Bangert**
Biological Sciences
Idaho State University
Pocatello, ID

**Robert C. Barbour**
Cooperative Research Centre for Sustainable Production Forestry
School of Plant Science
University of Tasmania
Australia

**Kelly R. Barr**
Department of Biology
University of Louisiana
Lafayette, LA

**Anbreen Bashir**
Department of Biology
St. Louis University
St. Louis, MO

**Dario Beraldi**
Wild Evolution Group
Institute of Evolutionary Biology
School of Biological Sciences
University of Edinburgh
Edinburgh, UK

**John W. Bickham**
Department of Forestry and Natural Resources and Center for the Environment
Purdue University
West Lafayette, IN

**Joseph D. Busch**
Microbial Genetics and Genomics Center
Northern Arizona University
Flagstaff, AZ

**Robert H. Devlin**
Fisheries and Oceans Canada
West Vancouver, BC
Canada

**J. Andrew DeWoody**
Departments of Forestry and Natural Resources and Biological Sciences
Purdue University
West Lafayette, IN

**Alexis Ducousso**
UMR Biodiversité Gènes et Communautés
Institut National de la Recherche Agronomique
Cestas, France

**Yousry A. El-Kassaby**
Faculty of Forestry
University of British Columbia
Vancouver, BC
Canada

**Julie R. Etterson**
Department of Biology
University of Minnesota Duluth
Duluth, MN

**Luke M. Evans**
Department of Biological Sciences
Northern Arizona University
Flagstaff, AZ

**Dylan G. Fischer**
The Evergreen State College
Olympia, WA

**Catherine A. Gehring**
Department of Biological Sciences
Northern Arizona University
Flagstaff, AZ

**Jake Gratten**
Department of Animal and Plant Sciences
University of Sheffield
Sheffield, UK

**Matthew C. Hale**
Department of Biological Sciences
Purdue University
West Lafayette, IN

**J. L. Hamrick**
Department of Plant Biology
University of Georgia
Athens, GA

**Richard G. Harrison**
Department of Ecology and Evolutionary Biology
Cornell University
Ithaca, NY

**David M. Hillis**
Section of Integrative Biology
University of Texas
Austin, TX

**Jeffrey D. Holland**
Department of Entomology
Purdue University
West Lafayette, IN

**Rebecca M. Holmstrom**
Department of Biology
University of Minnesota Duluth
Duluth, MN

**Rodney L. Honeycutt**
Natural Science Division
Pepperdine University, Seaver College
Malibu, CA

**Jamie A. Ivy**
Department of Collections
San Diego Zoo
San Diego, CA

**Sansanee Jamjod**
Faculty of Agriculture
Chiang Mai University
Chiang Mai, Thailand

**Antoine Kremer**
UMR Biodiversité Gènes et Communautés
Institut National de la Recherche Agronomique
Cestas, France

**Robert C. Lacy**
Department of Conservation Science
Chicago Zoological Society
Brookfield, IL

**Richard F. Lance**
Environmental Lab
U.S. Army Engineer Research and Development Center
Vicksburg, MS

**Emily K. Latch**
Department of Biological Sciences
University of Wisconsin–Milwaukee
Milwaukee, WI

**Peter W. Lawson**
Conservation Biology Division
NOAA Fisheries
Northwest Fisheries Science Center
Newport, OR

**Valérie Le Corre**
UMR Biologie et Gestion des Adventices
Institut National de la Recherche Agronomique
Dijon, France

**Paul L. Leberg**
Department of Biology
University of Louisiana
Lafayette, LA

**Carri J. LeRoy**
The Evergreen State College
Olympia, WA

**Wesley J. Leverich**
Department of Biology
St. Louis University
St. Louis, MO

**Denise L. Lindsay**
Environmental Lab
U.S. Army Engineer Research and Development Center
Vicksburg, MS

**Chanya Maneechote**
Faculty of Agriculture
Chiang Mai University
Chiang Mai, Thailand

**Marjorie Matocq**
Department of Natural Resources and Environmental Science
University of Nevada
Reno, NV

**Michelle M. McClure**
Conservation Biology Division
NOAA Fisheries
Northwest Fisheries Science Center
Seattle, WA

**Jennifer McCreight**
Department of Forestry and Natural Resources
Purdue University
West Lafayette, IN

**Paul McElhany**
Conservation Biology Division
NOAA Fisheries
Northwest Fisheries Science Center
Seattle, WA

**Allan F. McRae**
Genetic Epidemiology Group
Queensland Institute of Medical Research
Herston, Australia

**Charles H. Michler**
Department of Forestry and Natural Resources and Hardwood Tree Improvement and Regeneration Center
Purdue University
West Lafayette, IN

**David B. Neale**
Department of Plant Sciences
University of California at Davis
Davis, CA

**Krista M. Nichols**
Departments of Forestry and Natural Resources and Biological Sciences
Purdue University
West Lafayette, IN

**Sunisa Niruntrayakul**
Faculty of Agriculture
Chiang Mai University
Chiang Mai, Thailand

**Stephen R. Palumbi**
Department of Biology
Hopkins Marine Station
Stanford University
Pacific Grove, CA

**Josephine M. Pemberton**
Wild Evolution Group
Institute of Evolutionary Biology
School of Biological Sciences
University of Edinburgh
Edinburgh, UK

**Rémy J. Petit**
UMR Biodiversité Gènes et Communautés
Institut National de la Recherche Agronomique
Cestas, France

**Bradley M. Potts**
Cooperative Research Centre for Sustainable Production Forestry
School of Plant Science
University of Tasmania
Australia

**Amena Prommin**
Faculty of Agriculture
Chiang Mai University
Chiang Mai, Thailand

**Adirek Punyalue**
Faculty of Agriculture
Chiang Mai University
Chiang Mai, Thailand

**Tonapha Pusadee**
Faculty of Agriculture
Chiang Mai University
Chiang Mai, Thailand

**Benjavan Rerkasem**
Faculty of Agriculture
Chiang Mai University
Chiang Mai, Thailand

**Olin E. Rhodes, Jr.**
Department of Forestry and Natural Resources
Purdue University
West Lafayette, IN

**Kermit Ritland**
Department of Forest Sciences
University of British Columbia
Vancouver, BC
Canada

**Barbara Schaal**
Department of Biology
Washington University
St. Louis, MO

**Jennifer A. Schweitzer**
Ecology and Evolutionary Biology
University of Tennessee
Knoxville, TN

**Lee R. Shugart**
LR Shugart and Associates, Inc.
Oak Ridge, TN

**Theerasak Sintukhiew**
Faculty of Agriculture
Chiang Mai University
Chiang Mai, Thailand

**Jon Slate**
Department of Animal and Plant Sciences
University of Sheffield
Sheffield, UK

**Fredrik L. Sundström**
Fisheries and Oceans Canada
West Vancouver, BC
Canada

**Athitya Suta**
Faculty of Agriculture
Chiang Mai University
Chiang Mai, Thailand

**Chris W. Theodorakis**
Biology Department
Southern Illinois University at Edwardsville
Edwardsville, IL

**Peter M. Visscher**
Queensland Institute of Medical Research
Royal Brisbane Hospital
Queensland, Australia

**Thomas C. Wainwright**
Fish Ecology Division
NOAA Fisheries
Northwest Fisheries Science Center
Newport, OR

**Lisette Waits**
Fish and Wildlife Resources
University of Idaho
Moscow, ID

**Robin S. Waples**
Conservation Biology Division
NOAA Fisheries
Northwest Fisheries Science Center
Seattle, WA

**Peter M. Waser**
Department of Biological Sciences
Purdue University
West Lafayette, IN

**David J. Weston**
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN

**Thomas G. Whitham**
Department of Biological Sciences and Merriam-Powell Center for Environmental Research
Northern Arizona University
Flagstaff, AZ

**Alastair J. Wilson**
Institute of Evolutionary Biology
School of Biological Sciences
University of Edinburgh
Edinburgh, UK

**Keith E. Woeste**
Department of Forestry and Natural Resources and Hardwood Tree Improvement and Regeneration Center
Purdue University
West Lafayette, IN

**Anupong Wongtamee**
Faculty of Agriculture
Chiang Mai University
Chiang Mai, Thailand

**Lisa Worthen**
Department of Forestry and Natural Resources
Purdue University
West Lafayette, IN

**Stan D. Wullschleger**
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN

**Kelly R. Zamudio**
Department of Ecology and Evolutionary Biology
Cornell University
Ithaca, NY

# Preface

The world would be a wonderful place if our natural resources (e.g., forests, fish, and wildlife) needed no management and conservation was not a concern. In a world with a global human population approaching 7 billion and where most developed nations overconsume these resources, however, conservation is a concern and management is necessary for sustainable use. Historically, natural resource management strategies were determined by the collection and interpretation of basic field data. Today, as challenges to the sustainability and conservation of our natural resources arise, managers often need data that cannot be acquired using conventional methods. For example, a natural resource manager might want to know the number of successful breeders in a population or if genetic variation was being depleted because of a management practice. Traditional field craft alone cannot directly address such questions, but the answers can be determined with some precision if the field work is coupled with modern molecular genetic techniques.

Molecules can enlighten us about biological attributes that are virtually impossible to observe in the field (Avise 2004). Parentage analysis is one such arena in which genetic data can inform management practices (DeWoody 2005), but there are a host of others. For example, molecular data have revealed deep evolutionary splits in stocks at one time thought to be homogeneous. This finding has concomitant management implications (Hoffman et al. 2006). Similarly, molecules can enlighten us about biologies that are virtually impossible to observe in the field, such as pollen flow (Hamrick, this volume) or the physiology of migration (Nichols et al. 2008).

Recent advances in molecular genetics and genomics have been embraced by many scientists in natural resource conservation. Today, several major conservation and management journals (e.g., *Journal of Wildlife Management, North American Journal of Fisheries Management, Plant Breeding Reviews*) are now using "genetics" editors to deal solely with the influx of manuscripts that employ molecular data. We have attempted to synthesize some of the major uses of molecular markers in natural resource management in a book targeted not only at scientists but also at individuals actively making conservation and management decisions. To that end, we have identified contributors who are major figures in molecular ecology and evolution; many have published books of their own. Our aim has been to direct and distill the thoughts of these outstanding

scientists by compiling compelling case histories in molecular ecology as they apply to natural resource management.

Clearly, we hope this book will appeal to academics interested in conservation genetics, molecular ecology, and the quantitative genetics of wild organisms. We think this book could be used as an educational tool – as a text for graduate ecology/genetics courses but also, perhaps, in advanced undergraduate courses. Furthermore, we hope this book will be useful to audiences in natural resource management, education, and research by clarifying how genetic approaches can be used to answer resource-related questions.

## ABOUT THE EDITORS

Our collective expertise spans from molecular population genetics in the wild to genomics and quantitative genetics of managed or cultured species. We all study the genetics of natural resources, however, and we find that similar issues arise in wildlife, forestry, and fisheries. For example, when the forest geneticists began asking how many sires contributed pollen to a nut-bearing hardwood tree, it turns out that fisheries geneticists had already studied this problem from the perspective of a male fish guarding a nest full of developing embryos, and they had created computer programs to estimate the number of parents contributing gametes to a nest (DeWoody et al. 2000). Another such intersection of research across disciplines lies in the study of genetic processes in small populations; the same conceptual and analytical approaches being used to elucidate the genetic consequences of wildlife reintroductions (Latch & Rhodes 2005) are employed to evaluate genetic diversity in hardwood tree species subjected to severe habitat fragmentation (Victory et al. 2006). Our desire to produce a book stems from our mutual interests in understanding how molecular genetics can be used to inform and improve natural resource management.

In addition to our research interests, we teach several courses that directly pertain to this book. These courses include *Conservation Genetics* (DeWoody), *Molecular Ecology and Evolution* (DeWoody), and *Evolutionary Quantitative Genetics* (Nichols). Furthermore, several of us (DeWoody, Michler, Rhodes) have served as "genetics" editors for conservation and management journals, including *Journal of Wildlife Management, North American Journal of Fisheries Management*, and *Plant Breeding Reviews*.

## ACKNOWLEDGMENTS

In addition to the authors, most of whom also provided reviews on other chapters and/or boxes, we thank the following individuals for their invaluable feedback: Jean Beaulieu, Tasha Belfiore, John Burke, Dave Coltman, Tariq Ezaz, Ben Fitzpatrick, Anthony Fiumera, Mike Goodisman, Rick Howard, Irby Lovette, Bill Muir, Patty Parker, Devon Pearse, Joe Quattro, Kim Scribner, Ron Sederoff, and Rod Williams. All provided insightful comments that directly strengthened

Book contributors at an October 2008 meeting, held at the John S. Wright Forestry Center (Purdue University). Row 1: Krista Nichols, Kelly Zamudio, Charles Michler, Yousry El-Kassaby, Tom Whitham, Jamie Ivy, Emily Latch, Lisette Waits, and Marjorie Matocq. Row 2: Lee Shugart, Dave Neale, Dave Hillis, John Avise, Andrew DeWoody, Robin Waples, Rodney Honeycutt, Paul Leberg, and John Bickham. Row 3: Kermit Ritland, Antoine Kremer, Stan Wullschleger, Keith Woeste, Peter Waser, Jim Hamrick, Gene Rhodes, and John Patton. Photo credit: Caleb D. Phillips. *See Color Plate I*.

individual chapters and boxes, and we trust that this book has been enhanced by their efforts.

This volume was largely possible because of the financial and logistical support of the Department of Forestry and Natural Resources at Purdue University. In particular, the department sponsored an October 2008 meeting at Purdue where many of the book contributors congregated for three days of scientific discourse and fellowship before finalizing their respective chapters or boxes.

Our own research programs have been supported by a variety of organizations, including the National Science Foundation (DeWoody, Bickham, Michler, Nichols), the U.S. Department of Agriculture (DeWoody, Michler, Nichols, Rhodes, Woeste), the State of Indiana (DeWoody, Michler, Rhodes), the National Oceanic and Atmospheric Administration (Bickham), the Great Lakes Fishery Trust (DeWoody, Nichols), and the U.S. Forest Service (Michler, Woeste). We thank them all for investing in science.

## REFERENCES

Avise JC (2004) *Molecular Markers, Natural History, and Evolution*. Sinauer, Sunderland, MA.

DeWoody JA (2005) Molecular approaches to the study of parentage, relatedness and fitness: practical applications for wild animals. *Journal of Wildlife Management*, **69**, 1400–1418.

DeWoody JA, DeWoody YD, Fiumera A, Avise JC (2000) On the number of reproductives contributing to a half-sib progeny array. *Genetical Research* (Cambridge), **75**, 95–105.

Hoffman JI, Matson CW, Amos W, Loughlin TR, Bickham JW (2006) Deep genetic subdivision within a continuously distributed and highly vagile marine mammal, the Steller's sea lion (*Eumetopias jubatus*). *Molecular Ecology*, **15**, 2821–2832.

Latch EK, Rhodes OE Jr (2005) The effects of gene flow and population isolation on the genetic structure of reintroduced wild turkey populations: are genetic signatures of source populations retained? *Conservation Genetics*, **6**, 981–997.

Nichols KM, Felip A, Wheeler P, Thorgaard GH (2008) The genetic basis of smoltification-related traits in *Oncorhynchus mykiss*. *Genetics*, **179**, 1559–1575.

Victory E, Glaubitz JC, Rhodes OE Jr, Woeste KE (2006) Genetic homogeneity in *Juglans nigra* (Juglandaceae) at nuclear microsatellites. *American Journal of Botany*, **93**, 118–126.

# 1 Biodiversity discovery and its importance to conservation

Rodney L. Honeycutt, David M. Hillis, and John W. Bickham

During the eighteenth, nineteenth, and early twentieth centuries, scientific inventories of biodiversity flourished as naturalists participated in expeditions throughout different geographic regions of the world (Köhler et al. 2005). These expeditions and the various journals produced by many prominent naturalists provided materials for extensive scientific collections as well as accounts of the habits and habitats of both plant and animal species. Charles Darwin and Alfred Russel Wallace were part of this tradition, and both were students of biodiversity. They chronicled their adventures in South America, the Malay Archipelago, the Galapagos Islands, New Zealand, and Australia as they discovered new species, described geology, and encountered various cultures (Darwin 1845; Wallace 1869). These adventures honed their observational skills, and their experiences culminated in their parallel proposals of the theory of biological evolution by means of natural selection. The biodiversity and natural environments encountered by Darwin and Wallace have been altered, and both habitats and species described in their journals have and are being impacted at a drastic rate. The yellow-bridled finch (*Melanodera xanthogramma*), noted by Darwin as "common" in the Falkland Islands, is now gone, and, as predicted by Darwin, the Falkland Islands fox or warrah (*Dusicyon australis*) went extinct in 1876 (Armstrong 1994). The Borneo forest harbors fewer *Mias* or orangutans, and it is unlikely that one would be allowed to collect specimens like Wallace describes (Wallace 1869). Even "pristine" regions, such as those seen by Darwin in Patagonia and the southwest Atlantic coast of Argentina, are still poorly understood, yet they are threatened by numerous human activities (Bortolus & Schwindt 2007).

Owing primarily to the fact that the probability of massive species extinction is inevitable, interest in an all-species inventory and the derivation of a Tree of Life has increased over the last two decades. In 1992, the systematics community in the United States, through funding by the National Science Foundation, organized a meeting to set an agenda for the upcoming millennium. As a consequence, Systematics Agenda 2000 (1994) established three major goals: 1) to conduct a worldwide survey and inventory of all species and the taxonomic description of new species; 2) to derive a phylogeny or Tree of Life for all species that would serve as the basis for a classification as well as a framework for other researchers in the life sciences; and 3) to develop an information retrieval system for managing data on biodiversity.

Although our knowledge of biodiversity on planet Earth has increased as a consequence of the endeavors of early naturalists and these new initiatives, we are still far from a complete census of all species, and many will go extinct before their discovery. Such an inventory is essential because it provides a baseline for understanding the stability of ecosystems and the impact of anthropogenic processes that may eventually result in our own demise.

This chapter relates specifically to the inventory of biodiversity as an important step for its conservation. The first section provides a general overview of the importance of biodiversity to society, presents a survey of its global distribution, and identifies groups and geographic regions threatened by human activities. The second section reviews our current knowledge of worldwide biodiversity in terms of its discovery and description and identifies groups that are poorly known. The third section discusses the future of inventorying biodiversity and reviews how molecular approaches and phylogenetic methods provide means for accelerating the overall processes of species discovery and the construction of the Tree of Life. Finally, we emphasize the importance of an information retrieval system that makes data on biodiversity accessible to the entire scientific community.

## BIODIVERSITY

### Why is biodiversity important?

*Biodiversity* is defined as "the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems" (Glowka et al. 1994). The importance of biodiversity and the need for its conservation worldwide cannot be overemphasized. Not only are diverse forms of organisms responsible for sustaining human populations, they also serve important roles in the maintenance of ecosystems.

Advances in human medicine have benefited directly from biodiversity (Bernstein & Ludwig 2008; Harvey 2008). For instance, species of bacteria discovered over the last forty years have helped minimize transplant rejection, provided antibiotics and antifungal drugs that help combat infections from harmful pathogens, and revolutionized molecular biology by providing thermostable DNA polymerases used in polymerase chain reaction (PCR), a procedure employed broadly in medical diagnostics. Other plant and animal species provide drugs useful for treating cancer and serve as model organisms for studying molecular processes associated with disease and neurological disorders. Approximately 50% of the most broadly used drugs were derived from natural resources (Bernstein & Ludwig 2008), and biodiversity continues to serve as an important resource for the development of clinically important pharmaceuticals and other health-related products (Harvey 2008).

Aside from medicine, humans receive both direct and indirect benefits from biodiversity through the provision of food, fuel, clean water, and fertile soil

that enhances agriculture. According to Wilson (1985), more than seven thousand species of plants have been used for food, and twenty species are essential as worldwide food sources. Related species to those currently used for food are genetic reservoirs containing genes that may serve as sources of resistance to pathogens and pests as well as to potential climatic changes. In fact, enhancing both genetic diversity and crop diversity to create more complex "agroecosystems" may provide a more natural means of not only increasing production but also reducing the need for excessive use of pesticides and other chemicals (Altieri 2004). Biodiversity also provides a host of benefits and services to ecosystems. Processes associated with the recycling of nutrients, carbon and nitrogen cycling, formation of soils, climate stabilization, plant pollination, and decomposition of pollutants are influenced by biodiversity, and many of these processes are important to worldwide economies (Pimentel et al. 1997).

## How is biodiversity distributed?

Biodiversity is not randomly distributed worldwide. Comparisons across biogeographic regions reveal areas that differ significantly in terms of species diversity and levels of endemism (Gaston 2000). Two major patterns associated with differences in biodiversity are considered relevant to conservation issues. First, species richness varies over a latitudinal gradient, with more species occurring in the tropics than in more temperate regions (Gaston 1996). This general pattern appears to hold for many different taxa, such as plants, mammals, and birds, yet there are exceptions. Although amphibians are more diverse in the tropics, their general pattern of diversity is not completely correlated with latitude in that they do show local exceptions, such as their high diversity in the mountains of the eastern United States relative to other areas of North America and Europe (Buckley & Jetz 2007). This latitudinal gradient associated with species richness also appears to be asymmetrical, with the gradient stronger in the northern than in the southern hemisphere (Chown et al. 2004). As indicated by several authors (Gaston 2000; Hawkins 2001; Ricklefs 2004; Buckley & Jetz 2007; Dyer et al. 2007), the mechanisms responsible for the latitudinal gradient are widely debated but probably relate to several different factors including ecological (e.g., species interactions), environmental (e.g., habitat quality, energy, and degree of stability), and historical processes (e.g., degree of isolation, rates of extinction, migration, and speciation). Second, species richness increases with size of area, and, like latitudinal gradients, species-area curves are a pattern observed for plants, animals, and bacteria (Rosenzweig 1995; Horner-Devine et al. 2004). According to Rosenzweig (1992), latitudinal gradients and species-area curves occur at different temporal and spatial scales, with the latter occurring recently and at a more local or regional scale. In terms of predicting species diversity, this area effect probably relates to habitat heterogeneity (Rosenzweig 1992). For instance, Horner-Devine and colleagues (2004) observed an increase in bacterial species diversity with an increase in area that appeared related to an increase in overall habitat heterogeneity as the distance between sites in a salt marsh increased, and Báldi (2008) found that arthropod diversity on several reserves varied in response to habitat heterogeneity rather than to area.

Regardless of the mechanisms for latitudinal gradients and species-area curves, both of these general observations have been used to establish priorities for maximizing the conservation of biodiversity through the identification of regions (termed "biodiversity hotspots") that should receive high conservation priority (Myers 1988). Two criteria are commonly used to identify biodiversity hotspots. First, the number of endemic species (i.e., species that cannot be replaced if lost from a region) is considered a more important indicator than species richness, which is potentially biased toward broadly distributed species. Second, areas with high levels of endemism and under threat of habitat loss receive the highest conservation priority. The overall establishment of biodiversity hotspots is based on an extrapolation of better-known species, especially those that are indicators of habitats. As such, plant diversity is a common means of ranking hotspots. For instance, some of the first hotspots (e.g., ten sites in tropical rainforests) were recognized based on plant diversity (Myers 1988). Similarly, Mittermeier and colleagues (1998) identified twenty-four biodiversity hotspots on the basis of plant species endemism and the degree to which vegetation cover was being removed (some as high as 98%). These original twenty-four hotspots represented approximately 2% of land surface and approximately 46% of endemic plant species (Mittermeier et al. 1998). Currently, Myers and colleagues (2000) recognize twenty-five terrestrial hotspots, encompassing 1.4% of the world's land area and representing a large percentage of plant and vertebrate species. As before, the primary indicator of these biodiversity hotspots is percentage of endemic plant species and secondarily the percentage of endemic species of mammals, birds, reptiles, and amphibians.

There is an inherent assumption that uniqueness of (primarily) plants and (secondarily) vertebrates, as indicators of hotspots, can be extrapolated to lesser-known taxa such as invertebrates. The establishment of global priorities of conservation based on this assumption is somewhat problematic. For instance, Grenyer and colleagues (2006) examined the distribution of three vertebrate groups (birds, mammals, and amphibians) and found similar species richness among regions, yet little congruence in terms of the identification of hotspots based on the distribution of rare and vulnerable species associated with each group. This finding suggests that setting global priorities on the basis of surrogate taxa may be inappropriate, especially when identifying smaller, regional areas for conservation activities (Reid 1998). The finding also implies that broader taxonomic coverage is required for the identification of hotspots that encompass the majority of rare and endemic species.

## Is the extinction of biodiversity a problem?

> Those living today will either win the race against extinction or lose it, the latter for all time. They will earn either everlasting honor or everlasting contempt.
>
> (E. O. Wilson 2006, p. 99)

Extant species represent approximately 1–2% of the Earth's historical biodiversity (May et al. 1995). Therefore, *extinction*, the loss of a lineage with no replacement, is a natural process that appears nonrandom, relative to the species

that go extinct, and "episodic" in the fossil record (Raup 1986, 1994). This pattern of extinction implies that the average life span for most species is short, between one and ten million years (May et al. 1995). Theoretically, the Tree of Life can withstand random and "vigorous pruning" and recover from major extinction events (Nee & May 1997). Therefore, if extinction is a natural process and the Tree of Life is capable of responding to large extinction events, why is extinction a major concern of persons and groups interested in the conservation of biodiversity? The answers are twofold. The first is from a selfish point of view: The composition of communities that will appear subsequent to such pruning is likely to be different. The loss of important ecosystem services necessary for human survival implies that *Homo sapiens* might be a casualty of rapid and random extinction processes. Even if biodiversity loss does not cause extinction of our species, it is sure to have profound negative impacts on our society. The second answer, of more immediate importance, is the estimated rate at which biodiversity is currently going extinct. Based on the fossil record, Earth has experienced five mass extinctions, each resulting in a net loss of 75–95% of species (Raup 1994). Although difficult to quantify, most evidence suggests that current rates of extinction may be approaching those experienced during mass extinctions. On the basis of annual loss from deforestation and International Union for Conservation of Nature (IUCN) listings, May and colleagues (1995) calculated a range of 200–500 years for the current life span of a species. In a separate study, the current rate of extinction was estimated to be 100–1,000 times faster than the rate estimated prior to humans (Pimm et al. 1995).

According to IUCN's Red List assessment (Baillie et al. 2004), the rate of extinction for birds, amphibians, and mammals over the last century is 50–500 times higher than background extinction. Since the 1500s, 884 extinctions (784 total extinctions and 60 extinctions in the wild) of all species assessed by IUCN have been verified (Baillie et al. 2004). The rate of extinction for amphibians, reptiles, and mammals has increased since the beginning of the twentieth century, whereas extinction of birds started increasing in the eighteenth century, especially on Oceanic islands (Nilsson 2005; Fig. 1–1).

Extinction is an ongoing process, and although many currently recognized species are not extinct, a large number are increasing in vulnerability to extinction. For instance, of the 44,838 species assessed by IUCN (2008), 38% are threatened with extinction, and, except for birds and mammals, the other vertebrate groups show an increased rate of addition to the threatened list between the years 1996 and 2008, owing primarily to an increase in the number of species assessed for these groups (Fig. 1–2). Nearly complete assessments of mammals, birds, and amphibians were performed, and a summary of results is shown in Table 1–1.

Most species of birds, mammals, and amphibians have been evaluated by IUCN, and, among these three groups of vertebrates, amphibians worldwide show the highest risk of extinction. As of January 2010, 6,603 living species have been described (AmphibiaWeb 2010). Table 1–1 shows the number of species considered by IUCN in 2008; approximately 32% are threatened with extinction (Wake & Vredenburg 2008), which represents a potential rate of extinction that may approach 45,000 times the background rate. In comparison to birds and

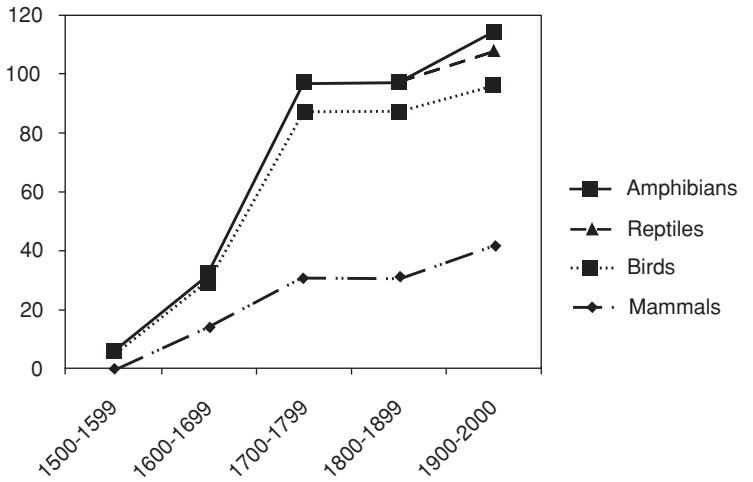



**Figure 1–1:** Extinction of vertebrate species between 1500 and 2000 (modified from Nilsson 2005).

mammals, twice as many species of amphibians are listed as critically endangered, and nearly one-third of the extinctions of amphibians have occurred in the last thirty years (Stuart et al. 2004). The current rate of amphibian decline has been referred to as "enigmatic" (Stuart et al. 2004) in that the processes responsible are complex, being caused by a host of potential culprits including fungal pathogens, loss of habitat, and changes in climate. The declines are not random. Amphibian communities in the neotropics, especially those in streams, are highly threatened (Dudgeon et al. 2006). In addition, of the 220 species of amphibians in Madagascar, 55 are threatened (Andreone et al. 2005).

A quarter of mammalian species (both marine and terrestrial forms) are vulnerable to extinction, and a high percentage of species show evidence of ongoing

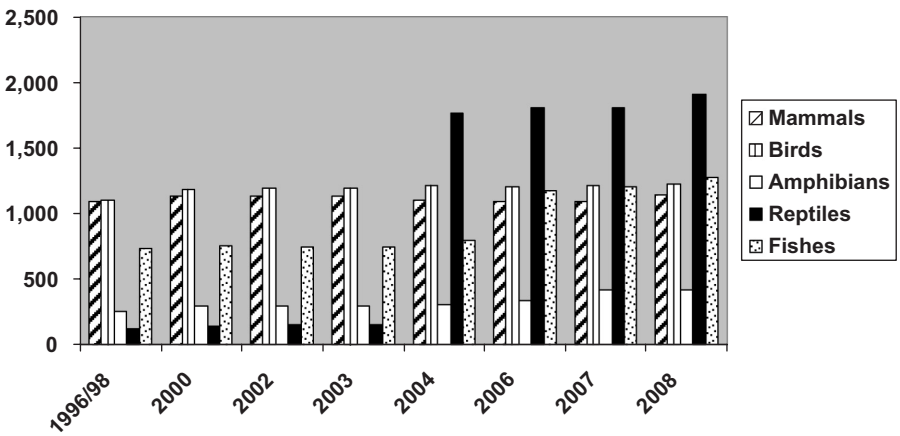



**Figure 1–2:** Changes in numbers of species of vertebrates in the threatened categories (critical, endangered, vulnerable) from 1996 through 2008 (derived from IUCN's Red List of Threatened Species™ 2008).

**Table 1–1.** Statistics on threatened species compiled from Table 1 of IUCN's Red List of Threatened Species™ (2008)

| | Described species | Evaluated by IUCN 2008 | Threatened | % Threatened based on number evaluated |
|---|---|---|---|---|
| Vertebrates | 61,259 | 26,604 | 5,966 | 22 |
| Mammals | 5,488 | 5,488 | 1,141 | 21 |
| Birds | 9,990 | 9,990 | 1,222 | 12 |
| Reptiles | 8,734 | 1,385 | 423 | 31 |
| Amphibians | 6,341 | 6,260 | 1,905 | 30 |
| Fish | 30,700 | 3,481 | 1,275 | 37 |
| Invertebrates | 1,232,384 | 6,161 | 2,496 | 41 |
| Insects | 950,000 | 1,259 | 626 | 50 |
| Molluscs | 81,000 | 2,212 | 978 | 44 |
| Crustaceans | 40,000 | 1,735 | 1,735 | 35 |
| Corals | 2,175 | 856 | 235 | 27 |
| Arachnids | 98,000 | 32 | 18 | 56 |
| Others | 61,209 | 67 | 33 | 49 |
| Plants | 298,506 | 12,055 | 8,457 | 70 |
| Lichens, Mushrooms, Brown Algae | 50,040 | 18 | 9 | 50 |
| Total | 1,642,189 | 44,838 | 16,929 | 38 |

population decline (Schipper et al. 2008). The trend in mammalian declines is not random in that some regions and groups of species are at greater risk. For instance, nearly 80% of all species of primates from Southeast Asia are threatened (Schipper et al. 2008), and larger mammals in general are more at risk, especially those that have been or are being overexploited by humans, such as the African elephant and many carnivores. Populations of many species of great whales were reduced to near extinction by commercial whaling. Some species have recovered to some degree, but others, including the Atlantic right whale, the Spitsbergen and Okhotsk Sea stocks of bowhead whales, and the western Pacific population of gray whales, are critically endangered and have not recovered despite the cessation of commercial whaling (IWC 2007). There are multiple causes for the demise of mammalian diversity including loss of habitat such as tropical deforestation, overharvesting and bycatch, pollution, and climate change. The Yangtze River dolphin or baiji (*Lipotes vexillifer*) has been declared as extinct, and its extinction represents the first mega-vertebrate extinction in fifty years and the first human-caused extinction of a cetacean. Moreover, it is the fourth mammalian family to become extinct in 500 years (Turvey et al. 2007).

Relative to the total number of species, birds (at 14%) are the least threatened. Extinctions are better documented for birds than probably any other group, however, and the patterns and processes of avian extinctions merit discussion. Some of the more vulnerable regions for birds are islands where, historically, most avian extinctions have occurred. Today, nearly 40% of avian species listed as threatened occur on islands (Johnson & Stattersfield 2008) and have an extremely high probability of extinction relative to mainland species (Trevino et al. 2007). The causes of both extinction and increased vulnerability of island birds include loss of habitat, overexploitation by humans, and the introduction of invasive species

(Johnson & Stattersfield 2008). On some islands, bird diversity has been severely depleted as a result of one or more of these causes. For instance, on Guam, ten of the thirteen species of forest birds are now extinct as a result of the brown tree snake, a species accidentally introduced after World War II (Fritts & Rodda 1998). A large percentage of threatened birds, however, occur in forested mainland habitats, many of which are subject to deforestation and fragmentation (Brooks et al. 1999) that together are accelerating the probability of avian extinctions. One particular region that has experienced considerable loss of habitat is the Atlantic Forest of Brazil and Argentina. According to Ribon and colleagues (2003), approximately 60% of the bird species in this region are either extinct or vulnerable to extinction.

Thorough assessments of both reptiles and fishes are lacking, but both are threatened as a result of overharvesting and loss of habitat. For reptiles, the percentages of threatened chelonians (turtles and tortoises) and crocodilians are 42% and 43%, respectively (Baillie et al. 2004). Although fish diversity is poorly known relative to that of other groups of vertebrates, freshwater ecosystems in general are extremely threatened, and, according to Lundberg and colleagues (2000), approximately 40% (10,000) of all described species of fish occupy freshwater, which makes up 0.01% of the world's water. As indicated by Dudgeon and coworkers (2006), freshwater systems represent the "ultimate conservation challenge" as a result of increased use of this resource worldwide. This increased use threatens not only fish but also other vertebrates, invertebrates, and microbes that rely on freshwater habitats, and extinction rates may be five times higher than predicted for terrestrial ecosystems, reaching nearly 50% in North America (Ricciardi & Rasmussen 1999).

## ENUMERATION OF BIODIVERSITY

> We need an expedition to planet Earth, where probably fewer than 10 percent of the life forms are known to science, and fewer than 1 percent of those have been studied beyond a simple anatomical description and a few notes on natural history.
>
> (Wilson 2006, p. 116)

### Status of species discovery and description

Species represent the basic units by which biodiversity is measured, and, as such, the first goal of Systematics Agenda 2000 is critical. Accuracy in the estimation of extinction rates, the establishment of conservation priorities based on biodiversity hotspots, and the designation of lineages that are essential for ecosystem function and the long-term survival of biodiversity require knowledge of the approximate number of species currently inhabiting the Earth.

How far have we progressed in our discovery and description of species since Linnaeus? According to Mayr (1969), Linnaeus's *Systema Naturae* lists 4,162 species, and since Linnaeus's time, the enumeration of total species has shown progress, with the current number of discovered species being between 1.5 million

and 1.9 million (May 1988, 1990, 1992). Species discovery for birds (Mayr 1946; Monroe and Sibley 1990; Peterson 1998; MacKinnon 2000), mammals (May 1988; Wilson & Reeder 1993, 2005; Patterson 2001; IUCN Red List of Threatened Species 2008), amphibians (Glaw & Köhler 1998; Köhler et al. 2005; Frost 2006; AmphibiaWeb 2010), and turtles (Bickham et al. 2007) is reasonably well documented, and all these groups show an increase in species discovery since Linnaeus, with most species of birds described early in the last century (Fig. 1–3).

The overall rate of species discovery is clearly increasing. For instance, in 2006 (State of Observed Species 2008), 16,969 new species of plants and animals were discovered, with the majority represented by vascular plants and invertebrates (Fig. 1–4). The rate of discovery of amphibian species increased by approximately 26% between 1992 and 2003, and in some geographic regions (e.g., Madagascar) the increase was 42% (Köhler et al. 2005). Mammalian species continue to be discovered. According to Patterson (2000), the rate of discovery of mammalian species in the neotropics is ten times that seen for birds. This rate of discovery is especially high for smaller mammals, such as rodents and bats (Patterson 2001), and in some cases species were rediscovered from existing collections and more recent genetic studies (Patterson 2000).

Although microbial diversity is essential to ecosystem function (Woese 1994), only approximately 5,000 species have been described (Pace 1997). In the past, this lack of species discovery was hindered by the fact that approximately 99% of microbes cannot be cultured (Amann et al. 1995). Most knowledge of bacterial species diversity comes from nucleotide sequences of ribosomal ribonucleic acid (rRNA) (Pace et al. 1986), and molecular markers are now being used to survey microbial diversity in a variety of habitats including soil (Schloss & Handelsman 2006), air (Brodie et al. 2007), marine communities (Sogin et al. 2006; Frias-Lopez et al. 2008), and extreme environments (Huber et al. 2007).

Despite the overall increase in the rate of species discovery, the tally of all species is incomplete and varies greatly across groups. Previous estimates of the potential number of species range between 5 million and 50 million, and the most current estimates are between 15.6 million and 19 million species (Erwin 1982; May 1988, 1992, 1998; Hammond 1992; Stork 1993; Ødegaard 2000; Novotny et al. 2007). Therefore, based on these numbers, our knowledge is limited to about 10% of the Earth's biodiversity (Fig. 1–5). Even for some groups of vertebrates, an all-species inventory is far from complete. For instance, with the exception of turtles, which are reasonably well known and assessed (Baillie et al. 2004; Bickham et al. 2007), the conservation status of many species of reptiles and fishes is less well known, partially as a consequence of the lack of an all-species inventory for these two groups (Table 1–1).

The most species-rich groups of organisms are even less well known than reptiles and fishes (Fig. 1–5 and Table 1–1). Approximately 59% of all described species are insects and 75% of all described species are invertebrates, yet the conservation status for most of these species has not been evaluated (IUCN 2008; Table 1–1). Even more disturbing is the fact that 80–95% of insect species have not been discovered (Stork 2007), and the number of arthropod species may range between five million and thirty million (Erwin 1982; Ødegaard 2000). On the basis of molecular markers, we are far from determining the number of microbial

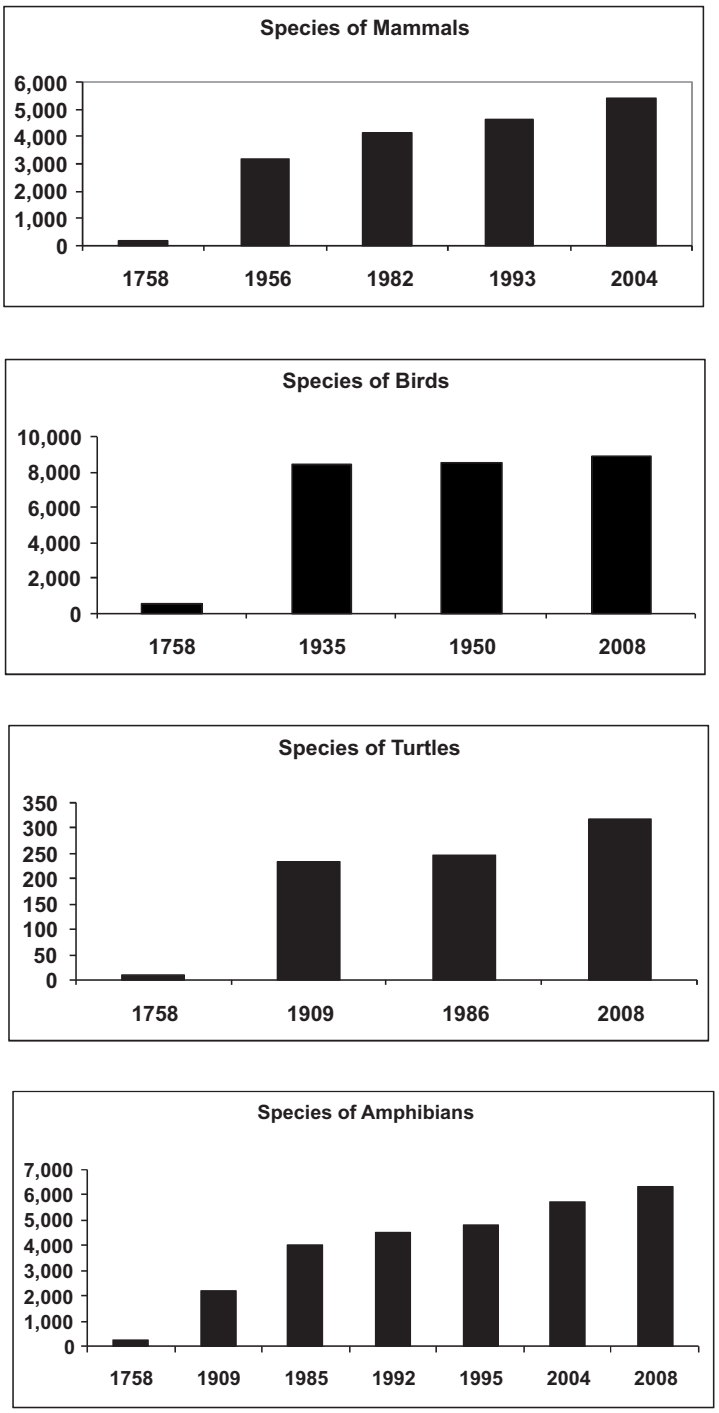



**Figure 1–3:** Species of vertebrates discovered since Linnaeus.

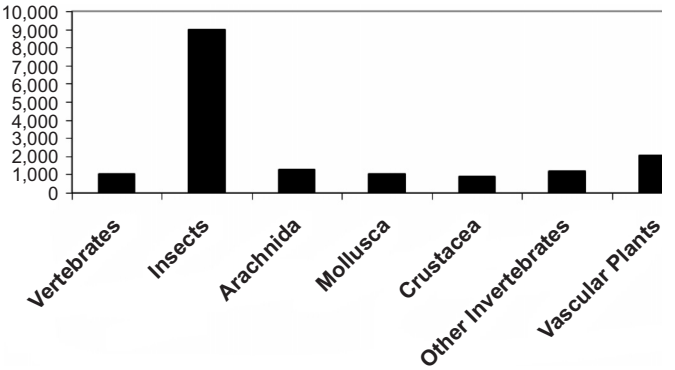



**Figure 1–4:** New species of plants and animals discovered in 2006 (modified from State of Observed Species 2008).

species, which may be 100 times higher than the number estimated using conventional techniques (Sogin et al. 2006).

### Factors limiting the rate of species discovery

Assuming that fifteen million species are undiscovered, the rate of discovery required to establish a complete inventory by the end of the century is approximately 150,000 per year, a rate more than 22 times higher than the average of the previous 250 years (approximately 6,800 species per year since the time of Linnaeus). The rate of species discovery in recent years has been higher than the 250-year average; as shown in Fig. 1–4, the current rate is approximately 17,000 species per year. Thus, the current rate of species discovery and description would need to increase approximately ninefold to reach fifteen million described species by 2100.

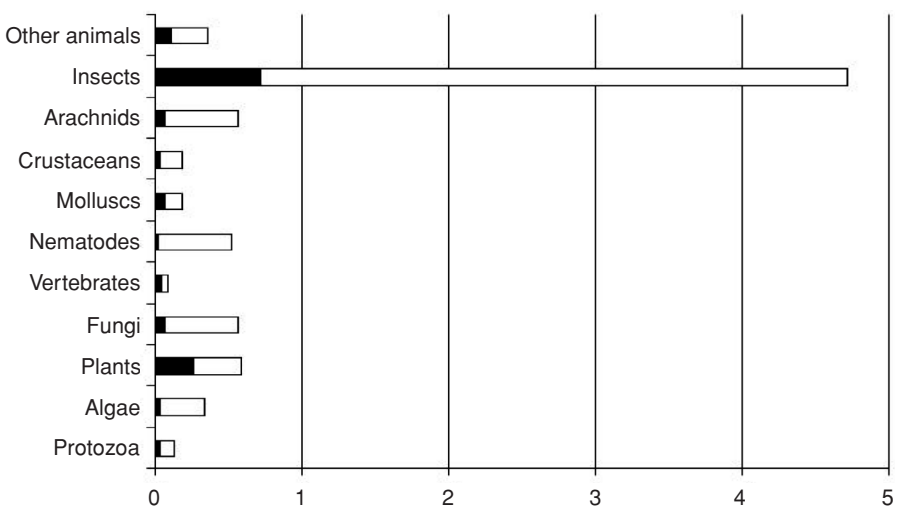



**Figure 1–5:** Described (black) and estimated number of extant species (white) from May (1998) with values in millions.

At this point, we have mostly described the easiest cases (large species in accessible places that can be distinguished morphologically), and much of the undiscovered biodiversity represents new challenges for systematists. Currently, several factors limit the feasibility of an all-species inventory. First, the discovery and description of new species, especially those in more diverse groups, require taxonomic expertise, and the number of experts varies greatly across groups. The slow pace of species descriptions is exacerbated by the loss of taxonomists familiar with some of the more diverse groups of taxa. This decline in the number of experts is unfortunate because studies of biodiversity rely on the accuracy of taxonomic descriptions and the establishment of a formal classification, both of which serve a "utilitarian" role for the identification and enumeration of species (Mayr 1969; Dubois 2003). As indicated by Wheeler (2004), the "infrastructure of taxonomy" needs to be reestablished through the funding and training of taxonomists.

Second, detailed surveys and inventories of geographic regions harboring large numbers of species are lacking. Decline of species diversity in these regions is increasing as a result of the alteration of natural habitats (Gibbons et al. 2000; Dudgeon et al. 2006). The process of discovery requires detailed assessments of geographic regions, some parts of which (e.g., North America, Europe, Japan, and Australia) are better known than others. Even though 32% of the described species of reptiles occur in the neotropics, less is known about the basic biology of many species (Urbina-Cardona 2008). Advances are being made to determine the status and distribution of reptiles worldwide (Cox et al. 2006), and a Global Biodiversity Assessment was started by IUCN in 2004. Regions of the world with high diversity of fishes are also poorly known, with the distribution of most species not well defined and diversity poorly surveyed (Lundberg et al. 2000; Dudgeon et al. 2006; Abell et al. 2008). As noted by Dudgeon and colleagues (2006), new species were being discovered at an average of several hundred per year between 1976 and 1994. Recently, existing information for more than 13,400 freshwater fish species was used to assign species to ecoregions characterized by level of endemism and diversity (Abell et al. 2008). More than half of these species are endemic to a specific ecoregion. Some of the highest species richness is observed in regions of Africa, the Amazonian Basin in South America, and parts of Asia. As indicated by these authors, some designated regions are "data poor." Nevertheless, this global assessment is a first step toward establishing conservation priorities for freshwater fish species.

Finally, the rate at which detailed species inventories are conducted needs to be increased, and technological advances in molecular genetics, especially those related to nucleotide sequencing, offer the best opportunity for accelerating the rate at which new taxa are discovered and placed within a phylogenetic context. The discovery of microbial species and their relationships has definitely benefited from the application of molecular techniques. For instance, phylogenies derived from rRNA sequences are the bases for a classification scheme that recognizes three major domains of life (Bacteria, Archaea, and Eukarya) as well as thirty or more major clades (Woese 1987; Pace 1997). In addition, the rate of discovery of new microbial species remains high, with considerable differences in species richness in different types of habitats (Schloss & Handelsman 2004). For

instance, in thirty grams of soil from forest habitat at least 500,000 species were found (Dykhuizen 1998), and in a relatively well-known habitat like the Sargasso Sea, 148 new species or phylotypes were discovered, suggesting that the actual diversity may be as high as 47,000 species (Venter et al. 2004). Recent surveys of deep sea vents also revealed 2,700 phylotypes of Archaea and 37,000 Bacteria (Huber et al. 2007), and in a recent study of the microbial ecology of human skin, 15 undescribed species were discovered (Gao et al. 2007).

Is it possible to accomplish this goal of a relatively complete assessment of the Earth's biodiversity in the twenty-first century by following traditional approaches employed by systematics? We argue that new technology and approaches, changes in taxonomic practice and culture, and a sustained increase in funding and training are needed to reach this goal. In the following section, we will discuss some of these issues as they relate to a total biodiversity inventory.

## FUTURE INVENTORY OF BIODIVERSITY

As indicated by Mayr (1969, p. 9), "The ultimate task of the systematist is not only to describe diversity of the living world but also to contribute to its understanding." The discipline of systematic biology is dedicated to the study of organic diversity, and the overall processes responsible for that diversity. The two major subdisciplines of systematics are phylogenetics and taxonomy. Taxonomy is required for the discovery, description, and identification of species, and the disciplines of taxonomy and phylogenetics merge in the creation of a classification scheme that reflects phylogenetic relationships. Formal names and recognized categories provide a mechanism for information retrieval that allows for the cataloguing and identification of worldwide biodiversity. Procedures used in classical taxonomy provide universal access to and communication about this information, and, as such, systematics in general should play a central role in the discovery and conservation of biodiversity. In fact, historical records derived from floras and faunas of various regions of the world and museum records provide the baseline for current information used to designate biodiversity hotspots and to assess the number of species threatened with extinction.

Although we feel that traditional systematics is essential to any enterprise designed to both discover and name all species on Earth, there are tools available for enhancing the efficiency and accuracy of species inventories. "DNA-based technology," improved phylogenetic methods, and databases that are Web-based and open access provide the necessary infrastructure for a concerted effort to survey and inventory all existing life forms on this planet. In the following sections, we will address some of these new technologies, including their appropriate contribution to the overall goal of the conservation of biodiversity and the completion of an all-species inventory.

### Importance of phylogenetics to the delimitation and conservation of species

> As buds give rise by growth to fresh buds, and these, if vigorous, branch out and overtop on all a feebler branch, so by generation I believe it has been with the

> Tree of Life, which fills with its dead and broken branches the crust of the earth, and covers the surface with its ever branching and beautiful ramifications.
>
> (Charles Darwin, 1859, Chapter 4)

Phylogenetics is sometimes poorly appreciated by many persons interested in the conservation of biodiversity and the inventorying of species. Nevertheless, Darwin's concept of descent with modification is graphically represented by a phylogeny that displays ancestor–descendent relationships and retains information about the overall pattern of biological diversification and extinction through time. As such, a phylogeny is an interpretive framework that serves several roles in conservation biology, including: 1) the delimitation of species based on the application of the phylogenetic species concept (PSC); 2) the identification of units of conservation, sometimes below the level of species; 3) the establishment of conservation priorities based on the diversity, age, and distribution of lineages; 4) the estimation of rates of speciation and extinction; and 5) the investigation of processes (e.g., climate, geology) that have influenced the historical and recent distribution and diversification of organisms.

The most obvious focal point in biodiversity science is the species because it is perceived as a real entity among biologists and is broadly appreciated by conservation biologists and the lay public. Species are also focal points for legal protection at the state, national, and international levels. Despite the significance of species in biodiversity conservation, the criteria used to delimit species are varied and sometimes contradictory, resulting in a diversity of species concepts (Mayden 1997). Studies of worldwide biodiversity require a method for delimiting species that is operational across a broad array of taxa, both sexually and asexually reproducing. According to Sites and Marshall (2003), many of the methods used in conservation biology can be subdivided into either non–tree-based or tree-based approaches. Barcoding, for instance, is a non–tree-based method that relies on magnitude of divergence as the major criterion for recognizing a species. Other non–tree-based methods emphasize either a lack of gene flow between populations or the grouping of individuals based on a set of distinguishing characteristics. In contrast, PSC criteria for tree-based methods emphasize monophyly as diagnosed on a phylogeny by shared-derived characters. This latter criterion is being broadly applied in conservation biology (Baker et al. 1995; Cracraft et al. 1998; Cracraft 2002; Wilting et al. 2007).

Application of the PSC as the major criterion for delimiting species does have consequences. In many cases, adherence to the PSC may result in higher numbers of species delimited (Agapow et al. 2004). This result is in part a consequence of recognizing subunits in a polytypic species that encompass several subspecies. Although the PSC is highly operational for delimiting species, it is stringent in its emphasis on monophyly. Part of the species problem is a consequence of the speciation process, which is a continuum with the level of divergence between lineages related to time since divergence (de Queiroz 2005). Because it is a continuum, lineages can be on different evolutionary trajectories without being monophyletic or reproductively isolated, and strict application of the PSC may result in a failure to identify unique yet recently diversifying lineages (Hey 2001). Despite this concern, however, the PSC provides a level of functionality that is useful for the discovery and delimitation of species, especially when one

of the major goals of biodiversity research is to determine the number of species inhabiting our planet. Additionally, many cases of species based on phylogenetic criteria are congruent with other criteria used to designate species, and phylogenetic discontinuities provide a meaningful way to reflect units of conservation (Avise & Walker 1999).

Ryder's (1986) idea of an "evolutionary significant unit (ESU)" represents an attempt to more objectively identify units of conservation that do not rely solely on traditional taxonomic designations, especially recognized subspecies. He emphasized agreement among multiple sources of data including distribution, ecology, morphology, and genetics (see also Chapter 10 by Waples and colleagues). In contrast, Moritz (1994) emphasized the need for a more "operational" definition of ESU based on the genetic and phylogenetic distinction of particular groups. His two specific criteria for ESUs included the diagnosis of "reciprocally monophyletic" groups identified from mitochondrial gene trees and evidence for "significant divergence of allele frequencies at nuclear loci." According to Moritz (1994), this definition captures evolutionarily distinct groups that result from historical processes. A second category introduced by Moritz (1994) was the management unit (MU), which is defined as a group that fails to show reciprocal monophyly but does reveal genetic divergence at either the mitochondrial locus or nuclear loci. Presumably, reduced gene flow identified for MUs reflects more recent events. Operationally, the criteria for ESUs are similar to the PSC and would essentially have the same consequence in terms of designating units of conservation. One interesting point raised by Moritz (1994) is the use of phylogeographic concordance among several species as a means of identifying regions that should receive high conservation priorities. In effect, this approach is similar to that of the hotspot, except that it focuses on areas related to patterns of geographic variation within species.

Moritz's criteria for recognizing both ESUs and MUs have been criticized for several reasons. First, some species will not show reciprocal monophyly yet still have populations that are demographically subdivided. This situation is especially problematic for recently separated species or ESUs that have not undergone lineage sorting (Avise 2000). The paraphyletic association between brown bears and polar bears, two groups that are morphologically and ecologically considerably different, provides an example of how the concept of reciprocal monophyly depicted in a mitochondrial gene tree may result in an incorrect decision about the designation of an ESU (Paetkau 1999). Second, some species may show high levels of geographic subdivision, thus resulting in the recognition of high numbers of ESUs distinguished by reciprocal monophyly. Third, if the concept of an ESU is analogous to that of a phylogenetic species, then formal taxonomic recognition may be preferred (Cracraft et al. 1998).

DeSalle and Amato (2004) subdivide genetic methods for the recognition of ESUs into two categories, a tree-based method and a "diagnostic character-based" method. The tree-based approach is best represented by the methodology introduced by Moritz (1994), which suggests that reciprocal monophyly defined by mitochondrial DNA (mtDNA) data and evidence of restricted nuclear gene flow are objective criteria for recognizing ESUs. The approach based on diagnostic characters does not require a gene tree but rather a collective set of substitutions that are diagnostic for a particular population (e.g., a suite of nucleotide substitutions

unique to a population or lineage). As indicated by DeSalle and Amato (2004), such an approach alleviates problems of gene trees failing to diagnose species trees.

Rather than setting conservation priorities based on endemism, vulnerability alone, or value based on esthetic, economic, or ecologic criteria, phylogenetic information provides a potential means of establishing priorities. A phylogeny not only depicts relationships among species but also provides estimates of amounts of divergence along lineages. In this light, branch lengths and branching patterns in a phylogeny provide a measure of the amount of evolution or genetic divergence that has occurred between species over time. Nearly all approaches designed to use phylogenies for setting conservation priorities establish criteria for ranking particular lineages. Such approaches have the potential of maximizing clade diversity and spread among clades throughout the phylogeny (Linder 1995). Vane-Wright and colleagues (1991) proposed an index that measures taxonomic distinctiveness. This particular approach combines information on both phylogeny and geographic distribution for the ranking of areas of biodiversity. In their approach, terminal taxa of a group receive less weight than more basal lineages that have little species diversity but display more evolutionary history as seen by their placement in a phylogeny. For instance, phylogenetic analyses of the freshwater fish fauna in Madagascar (a major biodiversity hotspot) reveal a large number of "basal taxa" that appear to be geographically localized and vulnerable to extinction. Therefore, the high level of endemism and large amount of evolutionary history make Madagascar a "reservoir of phylogenetic history" for freshwater fishes (Benstead et al. 2003).

Isaac and colleagues (2007) introduced the evolutionarily distinct and globally endangered (EDGE) score to identify species that should receive top conservation priority. In this approach, a species-level phylogeny is used as the interpretive framework for quantifying the overall score of a species. First, evolutionary distinctiveness (ED) is determined by calculating a value for each branch (length divided by number of species delimited by the branch) followed by the summation of all values from the base of the phylogeny to the terminal taxon of interest. Second, risk of extinction (GE) of a particular taxon is quantified based on the IUCN Red List category weight, and this value is combined with ED to provide an overall EDGE score. This particular method of setting conservation priorities was tested for a species-level phylogeny of mammals, and the results indicated that nearly half of the mammalian species with high EDGE scores did not coincide with current conservation priorities. This finding implies that if these taxa do not receive higher priority, the class Mammalia will lose a large portion of its phylogenetic diversity as estimated by EDGE scores.

Faith (1992) proposed a measure termed *phylogenetic diversity* (PD), which represents the summation of all branch lengths associated with a particular set of taxa in a phylogeny. Rather than focusing on species, this approach emphasizes the maximization of phylogenetic variance, as revealed by increasing levels of PD, and priorities of overall geographic regions or localities can be established based on the overall level of phylogenetic diversity associated with regions rather than estimates of either species richness or endemism.

Do the twenty-five currently recognized biodiversity hotspots capture a large majority of PD? Phylogenies for both primates and carnivores were used to

estimate the amount of evolutionary history or PD represented by the currently designated hotspots (Sechrest et al. 2002). The measure of both "clade evolutionary history" (sum of branch lengths of groups of species occurring in a particular area) and "species evolutionary history" (branch length associated with a species back to its most recent bifurcation) is in millions of years, as estimated using a molecular clock and branch lengths derived from a species-level phylogeny. The results of this approach indicated that hotspots exclusively contain one-third of the evolutionary history of these two groups. Therefore, although the establishment of conservation priorities based on habitat and level of endemism has been criticized, estimates of PD based on phylogenies of primates and mammalian carnivores indicate that these designated hotspots capture a considerable amount of PD and evolutionary history for these two groups.

Although a phylogenetic approach for delimiting species and the establishment of a natural classification are well justified in research related to biodiversity, the establishment of conservation priorities based on phylogenies is more tenuous. One must assume that not all species can be saved from extinction, and it is likely that many will go extinct before being discovered. It is also true, however, that the ability to pick lineages with an evolutionary future is impossible. For instance, the removal of one lineage during the history of the mammalian radiations could have resulted in the elimination of our species. Who would have predicted that this lone lineage would be so successful at exploiting our planet?

### Molecular taxonomy and phylogenetics of prokaryotes

In terms of species identification and discovery, microbes provide an excellent example of how molecular techniques can enhance the study of species diversity in a group that presents special difficulty with respect to culturing individual taxa. New molecular approaches have greatly expanded our knowledge of worldwide microbial diversity and have helped establish criteria for the recognition of species of bacteria (Ward 2002).

A distance-based approach represents one of the more traditional means of recognizing species of bacteria. For whole-genome comparisons, based on DNA/DNA reassociation, lineages that have 70% or greater similarity are considered strains within a species, whereas lineages less than 70% similar are considered different species (Embley & Stackebrandt 1997; Goodfellow et al. 1997; Cohan 2002; Gevers et al. 2005). Likewise, estimates of genetic divergence based on 16S rRNA sequences also consider different lineages as species if they are 3% or more divergent. Some studies have even used levels of divergence (3% to differentiate species, 5% genera, etc.) to diagnose categories in bacterial taxonomy (Wayne et al. 1987; Embley & Stackebrandt 1997; Schloss & Handelsman 2004). Although this approach has proven useful for assessing bacterial diversity, some consider such a phenetic or distance-based approach to be arbitrary. For instance, divergence based on small fragments of the 16S rRNA gene results in unstable estimates of relationships among species, and hypervariable regions in this gene show varying degrees of divergence across groups (Embley & Stackebrandt 1997; Goodfellow et al. 1997).

In contrast to a distance-based method, a phylogenic approach relies on sequencing (often the 16S rRNA gene) followed by the identification and placement of phylotypes in a phylogeny produced with the use of existing sequences (many from species that can be cultured and characterized) as well as new unknown sequences (Curtis & Sloan 2004). This approach provides a means of assigning species to functional groups, thus allowing for an evaluation of bacterial communities from different habitats and geographic locations (Whitaker et al. 2003; Venter et al. 2004).

Does the 16S rRNA gene provide enough information about the recognition of bacterial species and the derivation of a molecular phylogeny? As indicated by both Cohan (2002) and Gevers and colleagues (2005), the recognition of species based on sequences from the 16S rRNA gene alone is problematic in that some ecologically divergent lineages may have similar 16S rRNA sequences. Therefore, these authors suggest the recognition of species based on criteria that include both "genetic cohesion" and "ecological distinction." The former criterion suggests that lineages of bacteria tend to form phylogenetic clusters that can be characterized both phenotypically and ecologically. Determination of ecological distinction requires the use of multiple loci, termed the "multilocus sequence analysis" (MLSA) by Gevers and colleagues (2005). Phylogenetically distinct clusters, defined by either unique combinations of genes or patterns of gene expression (characteristics that suggest functional differences or ecological distinction), are considered different species. Such an approach is straightforward for strains that can be cultured and characterized in terms of their genome organization and ecological uniqueness. In contrast, species that cannot be cultured are difficult to characterize. In such cases, decisions as to whether an unknown sequence represents a new species or strain depend on its placement relative to well-characterized forms. Alternatively, shotgun sequencing and genome assembly, such as that performed by Venter and colleagues (2004), may provide a means of discovering new species of bacteria based on the criteria of genetic cohesion and ecological distinction.

### Molecular taxonomy and phylogenetics of eukaryotes

As with microbes, molecular markers are widely used to discover species and to diagnose phylogenetic relationships in eukaryotes. "The Barcode of Life" is a relatively recent idea that is based on the use of short sequences (650 bp) of a mitochondrial gene (cytochrome oxidase I or *cox*1) as a taxonomic character for the identification and potential discovery of species across broad taxonomic categories (Hebert et al. 2003a,b; Hajibabaei et al. 2007). The basic procedure is as follows: 1) The *cox*1 fragment is PCR amplified and sequenced from DNA obtained from an unknown specimen. 2) The sequence is then compared against a database containing sequences from previously identified taxa. 3) Criteria are established for either the identification of a particular unknown species relative to an existing species or the discovery of a new species.

Like the more traditional approach used for microbes, DNA barcoding is a distance-based approach that assigns specific cutoffs for species-level differences. The approach appears most effective at species identification, and, as such, it

provides valuable information for the identification of cryptic species and censuses designed to monitor invasive species. For instance, Hebert and colleagues (2004) used barcoding to distinguish among ten cryptic species of sympatric skipper butterflies, and the species identification was later confirmed with data from host plants, ecology, and color differences among caterpillars. Many invasive species gain entry as either larvae or forms at earlier stages of development, so identification can be difficult. Barcoding serves as an excellent means of identifying problematic invasive species (Savolainen et al. 2005).

Although extremely useful, the application of DNA barcoding does have some significant limitations, especially with respect to the general application for identification and discovery of species. First, *cox*1 is less appropriate as a marker for amphibian species in that intraspecific divergence can be high (7–14%) and can overlap with estimates of interspecific differences, and the primers suggested for amplification of the *cox*1 gene are not universal for amphibians (Vences et al. 2005). The latter problem, however, appears to be solved by modifying existing primers (Smith et al. 2008). For amphibians, a more effective molecular marker is the mitochondrial large subunit rRNA gene, which reveals less overlap between interspecific and intraspecific levels of divergence and is useful for diagnosing phylogenetic relationships among species (Vences et al. 2005). Likewise, different molecular markers appear more effective for not only species identification but also for the discovery of new species in plants. The database for *rbc*L gene sequences for plants is large, and this gene in combination with other loci (nuclear internal transcribed spacer [ITS] region and other chloroplast genes) provides an effective means of establishing phylogenetic relationships among taxa (Chase et al. 2005).

Second, the success of accurately identifying an existing species or discovering an undescribed species depends on the extensiveness of the existing database (Ekrem et al. 2007). Such databases are being assembled at GenBank and at the European Molecular Biology Laboratory (EMBL), and both organizations have established identifiers for searches of the barcode database. Nevertheless, these databases are limited by the existing numbers of species entries. Therefore, one problem with current searches of existing databases for the identification of either known or new species is that perfect matches may not occur (this is more likely when the databases are incomplete). There needs to be a concerted effort to increase sequence databases, especially for genes that are already being used for a broad number of species. For instance, the mitochondrial cytochrome *b* gene has been extensively examined for mammals (Bradley & Baker 2001). Therefore, mammalogists should make a concerted effort to enhance this database.

Third, barcoding currently lacks the ability to accurately place unknown specimens in a phylogenetic context. The derivation of an accurate phylogeny and the placement of unknown species in that phylogeny require the diagnosis of relationships among species and higher categories using an approach that emphasizes multiple genes and their products (DeSalle et al. 2005). The distance-based, single-gene approach used by barcoding can result in mistakes in the assignment of unknown specimens to particular groups (e.g., species complexes or genera) and can fail to identify the proper phylogenetic placement. This latter point is extremely problematic when one relies on data from a single mitochondrial gene rather than on independent data sources and overall congruence, especially

if the goal is to both discover new species and to accurately determine phylogenetic relationships among lineages. As more effective and accurate phylogenetic approaches and increased databases containing multiple gene sequences for species are developed, the overall accuracy of phylogenetic placement should be improved (Munch et al. 2008).

Finally, because mtDNA can be transferred between species through introgressive hybridization, the sole reliance on a mitochondrial marker may make it difficult to differentiate some taxa. MtDNA tracks maternal lineages, which in such cases do not reflect species lineages. One example is the North American deer of the genus *Odocoileus*, in which historical hybridization between mule deer (*O. hemionus*) and white-tailed deer (*O. virginianus*) has resulted in the establishment of a white-tailed deer mtDNA lineage within the mule deer. Nuclear markers, including Y-chromosomal sequences and allozymes, show a sister relationship between mule deer and black-tailed deer (both are *O. hemionus*), whereas mtDNA shows a sister relationship between mule deer and white-tailed deer (Carr et al. 1986; Cathey et al. 1998). In cases such as the North American deer complex, multiple genetic markers are required to resolve phylogenetic reticulations, and the use of genetic markers that track the four genetic transmission systems of mammals is an effective way to solve such evolutionary complexities (Lim et al. 2008; Trujillo et al. 2009).

### Molecular approaches to the discovery of cryptic species

Similar to some species of bacteria, the phenotypic characteristics of which are unknown as a result of their inability to be cultured, many groups of eukaryotes have species complexes that contain a number of cryptic species that are indistinguishable (or difficult to distinguish) at the phenotypic level. Like research on microbes, the advent of PCR and nucleotide sequencing has enhanced the ability to identify cryptic species, many of which are physiologically, behaviorally, or otherwise distinct, despite their morphological similarity. According to two recent surveys, articles dealing with the discovery of cryptic species based on molecular data are increasing exponentially, with between 2,235 and 3,500 articles reporting cryptic species published over the last two to three decades (Bickford et al. 2006; Pfenninger & Schwenk 2007). For the most part, if one corrects for differences in species richness, the discovery of cryptic species appears to be evenly distributed in terms of taxonomic groups and geographic distribution, with examples being found in a diversity of metazoan phyla (Fig. 1–6).

In many cases, broadly distributed species that are morphologically homogeneous throughout their range actually consist of several cryptic species. For example, bonefishes of the genus *Albula* have a pantropical distribution and traditionally have been considered a single species, *Albula vulpes*. Based on a detailed phylogenetic study of bonefishes throughout most of their range, as many as eight divergent lineages can be identified with the use of mitochondrial sequences. Many of these divergent lineages occur in areas of sympatry yet demonstrate no morphological distinction (Colborn et al. 2001) (see Box 1). The cosmopolitan species of moss *Grimmia laevigata* is morphologically similar throughout its broad distribution yet, based on amplified fragment length polymorphism (AFLP) data,

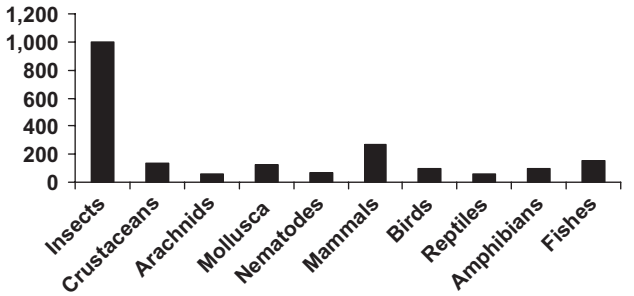



**Figure 1–6:** Number of reports of cryptic species between 1978 and 2006 (compiled from Pfenninger and Schwenk 2007).

the species in California consists of two cryptic species (Fernandez et al. 2006). Similarly, the sea star, *Parvulastra exigua*, a species broadly distributed in the southern hemisphere, consists of several cryptic species as defined on the basis of mtDNA divergence (Hart et al. 2006).

Amphibian diversity has been severely underestimated, partially due to the lack of morphological distinction among some forms and partially because so many species are narrowly endemic to small geographical areas. In Southeast Asia, the two broadly distributed species of frogs (*Odorrana livida* and *Rana chalconota*) actually represent as many as fourteen cryptic species, many of which are sympatric (Stuart et al. 2006). Given the rate at which habitat is being destroyed in this region of the world, such information is necessary for the proper identification of regions of endemism and the establishment of conservation priorities. Underestimates of amphibian biodiversity are not limited to Southeast Asia. Fouquet and colleagues (2007) used data from the mitochondrial 16S rRNA gene to examine frog diversity in the neotropics. On the basis of these molecular data, they identified twice as many candidate species (129) as the number of named species examined. Likewise, the frog *Eleutherodactylus ockendeni* in Ecuador probably represents at least three genetically distinct species (Elmer et al. 2007).

The problem of morphologically cryptic species has hindered research on some model organisms. For many decades, a single species of leopard frog, *Rana pipiens*, was thought to be distributed from Canada to Panama, throughout North and Middle America (Moore 1944). For much of the twentieth century, *Rana pipiens* was used extensively in research, especially in studies of physiology and endocrinology (Hillis 1988). As source populations for experimental animals changed, however, laboratory biologists who found different populations showed markedly different physiological responses. Studies of behavior (e.g., Littlejohn and Oldham 1968; Mecham 1971), reproductive timing (e.g., Hillis 1981; Frost & Platz 1983), and genetic compatibility (e.g., Moore 1975; Frost & Bagnara 1977) all indicated the existence of many species of biologically distinct, mostly cryptic species of leopard frogs throughout North and Middle America. Sorting out the cryptic species required extensive and careful analyses of behavioral, morphological, and genetic data, although the various species eventually proved readily distinguishable using analyses of proteins (Hillis et al. 1983) or DNA sequences (Hillis & Wilcox 2005). Although molecular studies of the phylogeny of the *Rana*

## BOX 1: GENETIC IDENTIFICATION OF CRYPTIC SPECIES: AN EXAMPLE IN *RHOGEESSA*

Amy B. Baird

### Problem

Understanding and describing the diversity of life on Earth is a daunting task. This problem is made especially difficult when species cannot be distinguished from one another based on traditional means. Cryptic species occur that are morphologically indistinguishable yet are genetically, behaviorally, or otherwise quite divergent. Biologists must take these differences into account when determining taxonomic status, as well as when planning conservation and management issues for the species of interest.

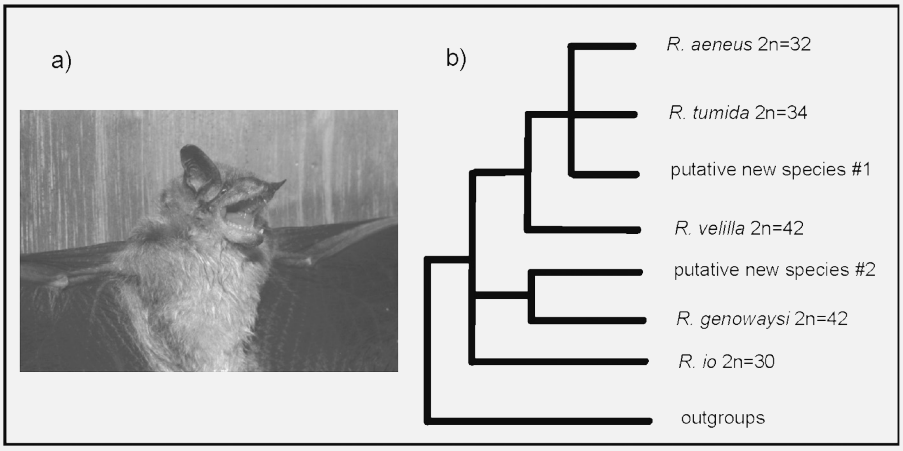
### Case Study

Cryptic species among mammals are relatively rare in some groups yet common in others (Baker & Bradley 2007). An example of a group of mammals that illustrates this is the bat genus *Rhogeessa*. Within the *R. tumida* complex, there are multiple species that are morphologically indistinguishable but were elevated to species status based on genetic differences (Box Fig. 1–1, a).

Historically, *Rhogeessa tumida* has been found from northern Mexico to northern South America. Chromosome banding studies performed within the last 30 years, however, have shown a high degree of karyotypic variation throughout this range (Bickham & Baker 1977). Allozyme analyses have confirmed that these various chromosome races were, in fact, genetically distinct groups (Baker et al. 1985), and they were later described as unique species based on these differences. Those species found to be karyotypically distinct were *R. aeneus*, *R. genowaysi*, and *R. io*.

Recent advances in DNA sequencing technology have allowed researchers to more accurately test the hypotheses of taxonomic status and degree of gene flow between members of the *R. tumida* species complex. By sequencing markers from mtDNA, Y-chromosomal DNA, and nuclear autosomal loci, researchers were able to confirm the taxonomic status of previously described members of the *R. tumida* complex (Baird et al. 2008, 2009). They showed that these species are genetically well differentiated and the molecular phylogenies are consistent with them being unique species (i.e., they are well-supported monophyletic groups; Box Fig. 1–1, b). These data also showed that with one possible exception (an ancient hybridization event between *R. tumida* and *R. aeneus*), the species in the *R. tumida* complex have been genetically isolated for a long period of time.

DNA data can often detect more subtle differences among populations than can karyotypic analyses. One surprising result of the molecular studies of the *R. tumida* complex was the finding of additional variation that did not correspond with karyotypic changes. These genetically distinct populations represent an additional two new species of *Rhogeessa* that are karyotypically identical to *R. tumida* (Baird et al. 2009). They also showed that a population of *Rhogeessa* in




**Box Figure 1–1:** (a) Image of one of the putative new species of *Rhogeessa*. (b) Phylogenetic relationships of members of the *R. tumida* species complex based on mtDNA sequences, modified from Baird and colleagues (2008). Note that branch lengths are not drawn to scale.

Ecuador, although karyotypically identical to *R. genowaysi*, was phylogenetically distinct based on mtDNA sequences and should be considered a separate species (named *R. velilla*). These results are significant because they were not predicted based on karyotypic or morphological analyses.

The case study of *Rhogeessa* highlights several important lessons for biodiversity studies. First, to understand the diversity of life on Earth, it is necessary to collect large amounts of DNA sequence data and analyze them in both phylogeographic and phylogenetic contexts. Second, efforts to conserve biodiversity should include an understanding of genetic variation so as to account for unknown cryptic species that might occur. This lesson is well illustrated by *Rhogeessa* because one of the cryptic species, *R. genowaysi*, is listed on the 2008 IUCN Red List as an endangered species due to habitat fragmentation and decline. This species is known only from a highly restricted range along the Pacific coast of Chiapas, Mexico, where the forests have been largely cleared for agriculture. Without genetic analyses, this species would never have been recognized; sadly, it might already be extinct.

## REFERENCES

Baker RJ, Bickham JW, Arnold ML (1985) Chromosomal evolution in *Rhogeessa (Chiroptera: Vespertilionidae)*: possible speciation by centric fusions. *Evolution*, **39**, 233–243.

Baker RJ, Bradley RD (2007) Speciation in mammals and the genetic species concept. *Journal of Mammalogy*, **87**, 643–662.

Baird AB, Hillis DM, Patton JC, Bickham JW (2008) Evolutionary history of the genus *Rhogeessa (Chiroptera: Vespertilionidae)* as revealed by mitochondrial DNA sequences. *Journal of Mammalogy*, **89**, 744–754.

Baird AB, Hillis DM, Patton JC, Bickham JW (2009) Speciation by monobrachial centric fusions: a test of the model using nuclear DNA sequences from the bat genus *Rhogeessa*. *Molecular Phylogenetics and Evolution*, **50**, 256–267.

Bickham JW, Baker RJ (1977) Implications of chromosomal variation in *Rhogeessa (Chiroptera: Vespertilionidae)*. *Journal of Mammalogy*, **58**, 448–453.

*pipiens* complex provide a rich context for comparative studies of the evolution of physiological and behavioral traits, the initial taxonomic complexity of this group forced many researchers to seek out and develop alternative model systems. In addition, much of the existing extensive literature on *Rana pipiens* is difficult to interpret because this name was used for so many decades to refer to many biologically distinct species. An earlier understanding of the species and relationships among the species in the *Rana pipiens* complex would have greatly facilitated the use of leopard frogs as experimental model organisms.

Molecular data are being used to discover new cryptic species of mammals and to modify the existing taxonomy of some well-known forms (Box 1). For instance, Brown and colleagues (2007) used genetic data to examine variation in the African giraffe, and found at least seven monophyletic lineages that probably represent distinct species. Recently, two species of African elephant (*Loxodonta africana* and *Loxodonta cyclotis*) have been recognized based on their genetic distinction at several nuclear gene loci (Roca et al. 2001). In the case of the elephants, the two species are not cryptic in that they do show some morphological differences. In other cases, molecular phylogenies derived primarily from mitochondrial sequences have been used to modify the existing taxonomy of mammals, either by relegating subspecies to specific-level status or rearranging existing subspecies boundaries. One case involves the Sumatran tiger (*Panthera tigris sumatrae*), which was designated as a species on the basis of its unique phylogenetic position relative to mainland forms (Cracraft et al. 1998). In this case, the authors used a PSC to justify this taxonomic change. Modification of subspecies boundaries of the common chimpanzee, *Pan troglodytes*, was recommended based on mitochondrial data that subdivided the three recognized subspecies into two monophyletic groups (Gonder et al. 2006).

The discovery of cryptic species is important to biodiversity research as well as to other areas of science. Identification of morphologically similar, yet genetically distinct, species is important to conservation efforts, especially if the establishment of conservation priorities is based on the uniqueness of particular lineages. Distinguishing cryptic species may result in partitioning patterns of endemism into finer spatial scales that are more conducive to conservation efforts, such as seen in Australian freshwater systems (Cook et al. 2008). Cryptic species also have implications for evolutionary biology in terms of understanding morphological stasis, speciation, ecological overlap, species recognition, host/race speciation, and many other topics. Finally, the recognition of cryptic species has applications in both medicine and agriculture, especially as it relates to the identification of human pathogens and plant pests and pathogens. Therefore, any detailed assessment of worldwide biodiversity will benefit from the use of genetic markers for identification and discovery of species. Without such an approach, our overall species count might be a severe underestimate.

## ENHANCING RATE OF SPECIES DISCOVERY

Taxonomic practice reveals that not all taxonomic characters are equally useful. Some are powerful indicators of relationship, others are not. The usefulness of a

> character depends on its information content, that is, on its correlation with the natural groupings of taxa produced by evolution.
>
> (Mayr 1969, p. 123)

Analyzing molecular characters in a phylogenetic framework offers a means of accelerating the rate of species discovery and identification, especially in groups containing either cryptic species or large numbers of species. Although molecular-based approaches are important for studying biodiversity, the application of traditional taxonomy is essential if our information databases are to be biologically sound and meaningful (Wheeler 2004).

Taxonomic databases derived from molecular markers exist for microbes, and newly developed molecular approaches have greatly increased the rate of species discovery and identification of microbial diversity. Application of these methods, combined with genomics, methods of sequence assembly, robotics, and the use of informational databases, has greatly increased overall estimates of microbial diversity worldwide. All of these approaches emphasize the acceleration of species identification and discovery with the use of high-throughput methods.

Some of these high-throughput methods used for studies of microbes have greatly accelerated the identification of microbial species, thus allowing for detailed studies of microbial diversity in different regions as well as the assessment of changes in diversity in response to environmental perturbations. For instance, microbial communities respond quickly to changes in the environment, yet assessing community response is hindered by the quantification of microbial diversity in both terrestrial and aquatic ecosystems. In marine ecosystems, the more traditional means of quantifying phytoplankton diversity, especially in terms of identifying species and genera, require microscopy. As indicated by Ellison and Burton (2005), identification via microscopy is more qualitative than quantitative, and even flow cytometric quantification is limited in the number of taxa that can be identified by photopigmentation. These authors have developed a method that uses DNA hybridization and bead-array technology for both the identification and quantification of species. This particular approach bypasses PCR amplification and instead assesses species diversity directly from whole DNA isolated from water samples. Taxon-specific probes containing different fluorescent tags attached to beads are hybridized to specific components of the total DNA. Flow cytometric techniques are then used for species identification and quantification, and the procedure accommodates screening on ninety-six well plates. Therefore, the method allows for rapid assessment of species diversity in different marine environments.

High-throughput methods are available for rapid assessment of bacterial diversity. Many of these methods are PCR-based and rely on assays of variation in the rRNA genes. Terminal restriction fragment length polymorphism (T-RFLP) is used to produce species-specific DNA fingerprints that can be analyzed on an automated sequencer (Schütte et al. 2008). The method uses total DNA extracted from a substrate (e.g., soil, water), fluorescently labeled primers, PCR amplification of the 16S rRNA gene, and digestion of the PCR product with specific restriction endonucleases. Existing databases of fingerprint profiles for particular species can be used to select restriction endonucleases and to identify species

by comparison to known taxa in the database (Marsh et al. 2000). Serial analysis of ribosomal sequence tags (SARST) and parallel pyrosequencing provide another high-throughput method for the rapid identification of species (Neufeld et al. 2004; Ashby et al. 2007; Huse et al. 2008). These methods amplify small hypervariable regions (17–55 base pairs) of the 16S rRNA gene from total DNA, and clones as many as twenty sequence tags in a single plasmid. Microarrays are used to sequence multiple plasmids, and particular sequence tags are used to identify taxa of microbes. This technique is cost effective and allows for high-throughput and rapid identification of components of a bacterial community. Although the size of sequenced fragments is limited, making them less reliable in a detailed phylogenetic study, these markers do allow for an assessment of bacterial communities as well as the discovery of rare components of the community.

A phylogenetic approach for species discovery of microbes relies on sequencing cloned amplicons (PCR amplification products) from 16S rRNA fragments amplified from total DNA extractions (Cottrell et al. 2005; Green & Keller 2006). This tree-based approach has been the "gold standard" for studying microbial biodiversity, and new high-throughput methods of DNA isolation, PCR amplification, cloning, and sequencing allow for hundreds of samples to be processed in a short period of time. This particular approach is essential because most species of microbes are known only from nucleotide sequences (Amann et al. 1995). Another more recent approach involves shotgun sequencing of sheared DNA cloned into specific vectors (Castiglioni et al. 2004; Venter et al. 2004; Schloss & Handelsman 2005; Tringe et al. 2005). Randomly obtained sequences are assembled into contigs and scaffolds. This "metagenomic approach" provides an effective means of species discovery in a large number of habitats.

In addition to the high-throughput methods for discovering microbial diversity, new technological advancements are making it possible to accelerate the discovery of eukaryotic species by several orders of magnitude. These molecular-based methods offer the ability to produce data in a format for rapid species identification and phylogenetic placement of unknown taxa. Most of these new devices use nanotechnology that provides platforms for rapid PCR amplification and sequencing. For instance, Blazej and colleagues (2008) describe a nanoliter-scale bioprocessor capable of all the steps necessary for sequencing including PCR, purification of PCR products, and capillary electrophoresis. This "lab-on-a-chip" device uses low amounts of DNA template and provides a means of sequencing more than 550 bp at high accuracy and low cost. Another instrument based on chip technology provides a means of PCR amplification and capillary analysis (Govind et al. 2003). Drmanac and colleagues (1998) also present a high-throughput technique, termed *sequencing by hybridization* (SBH), that uses labeled oligonucleotide probes (of known sequence) in replicate arrays that are hybridized to template DNA. As a result of ongoing technological advancements, it is not far-fetched to imagine a relatively inexpensive, handheld device that can isolate DNA, PCR amplify specific DNA fragments, rapidly sequence PCR products, and organize sequence data for immediate phylogenetic analysis and the screening of existing databases. Such a device could be carried into the field by biologists or other interested individuals and used to quickly identify

unknown species and to discover species that have never been previously identified by biologists. Such technology is needed if we have any hope of achieving a reasonably complete understanding of the biodiversity of the Earth in this century.

## PHYLOGENETIC DATABASES

> Imagine an electronic page for each species of organism on Earth, available everywhere by single access on command.
>
> (Edward O. Wilson, 2003, p. 77)

Identifying the name of an organism is of little utility by itself. The value in identifying an organism is that the name ties the organism to the scientific literature and other information about that species. Thus, the final and perhaps most important link between systematics and conservation is the establishment of effective, useful, and comprehensive databases on the diversity of life. Given that biological taxonomy is based on phylogenetic relationships, such databases need to be organized and searchable using phylogenetic information. In other words, when a biologist identifies an unknown as linked to a particular part of the Tree of Life (using, e.g., the methods described in the previous sections), he or she needs to be able to connect that organism with all the information on that species. If the unknown is a new species that has never before been studied, then the best information available will be the information on phylogenetically related species. This comparative framework is essential to the use and understanding of biodiversity resources.

There have been many recent efforts to develop effective systematic databases. Many of these are quite limited and amount to little more than lists of names, perhaps linked to bibliographic information on the original description. A more effective approach is to link all of the world's species with all of the information on those species. This is the idea behind the Encyclopedia of Life project (Wilson 2003; see http://www.eol.org). After the database is created, a biologist will be able to identify an unknown organism by placing it within the Tree of Life; this placement would automatically identify the species within the framework of biological taxonomy and immediately link the organism to the information on that (and related) species. Imagine the many and varied uses of such information, from human health applications to conservation biology, to bioprospecting for new useful compounds, to basic biological research. Suddenly, systematic biology would be a critical and necessary component of almost every interaction between people and the living world.

How will automated identification and phylogenetic databases make a difference to conservation biology? Our current ability to protect and understand biodiversity on Earth is severely hampered by our ignorance of what we are trying to preserve and study. If we only know about a small fraction of life on Earth, how can we possibly understand the function of ecosystems? At present, conservation biologists are like car mechanics, who are working to keep a car running, but who only have fragmentary knowledge about the function of 10% of the engine

parts. The other 90% of the parts are falling off the engine faster than they can be discovered, and it is unclear how much longer the car will keep running. In the case of the living world, systematics will help us identify and understand the various components of biodiversity, but only if biologists are willing to adopt new technologies and strategies to tackle the enormous undertaking that lies before us.

## REFERENCES


Abell R, Thieme ML, Revenga C et al. (2008) Freshwater ecoregions of the world: a new map of biogeographic units for freshwater biodiversity conservation. *BioScience*, **58**, 403–414.

Agapow P-M, Bininda-Emonds ORP, Crandall KA et al. (2004) The impact of species concept on biodiversity studies. *Quarterly Review of Biology*, **79**, 161–179.

Altieri, MA (2004) Linking ecologists and traditional farmers in the search for sustainable agriculture. *Frontiers in Ecology and the Environment*, **2**, 35–42.

Amann RI, Ludwig W, Schleifer KH (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiology Review*, **59**, 143–169.

AmphibiaWeb (2010) Information on amphibian biology and conservation. Berkeley, California, http://amphibiaweb.org.

Andreone F, Cadle JE, Cox N et al. (2005) Species review of amphibian extinction risks in Madagascar: conclusions from the global amphibian assessment. *Conservation Biology*, **19**, 1790–1802.

Armstrong PH (1994) Human impact on the Falkland Islands environment. *The Environmentalist*, **14**, 215–231.

Ashby MN, Rine J, Mongodin EF, Nelson KE, Dimster-Denk D (2007) Serial analysis of rRNA genes and the unexpected dominance of rare members of microbial communities. *Applied Environmental Microbiology*, **73**, 4532–4542.

Avise JC (2000) *Phylogeography: The History and Formation of Species*. Harvard University Press, Cambridge, MA.

Avise JC, Walker D (1999) Species realities and numbers in sexual vertebrates: perspectives from an asexually transmitted genome. *Proceedings of the National Academy of Sciences USA*, **96**, 992–995.

Baillie JEM, Hilton-Taylor C, Stuart SN, editors (2004) *2004 IUCN Red List of Threatened Species. A Global Species Assessment*. IUCN, Gland, Switzerland, and Cambridge, UK.

Baker AJ, Daugherty CH, Colborne R, Mclennani JL (1995) Flightless brown kiwis of New Zealand possess extremely subdivided population structure and cryptic species like small mammals. *Proceedings of the National Academy of Sciences USA*, **92**, 8254–8258.

Báldi A. (2008) Habitat heterogeneity overrides the species-area relationship. *Journal of Biogeography* **35**, 675–681.

Benstead JP, De Rham PH, Gattolliat J-L et al. (2003) Conserving Madagascar's freshwater biodiversity. *BioScience*, **53**, 1101–1111.

Bernstein AS, Ludwig DS (2008) The importance of biodiversity to medicine. *Journal of the American Medical Association*, **300**, 2297–2299.

Bickford D, Lohman DJ, Sodhi NS et al. (2006) Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution*, **22**, 148–155.

Bickham JW, Parham JF, Philippen H-D et al. (2007) An annotated list of modern turtle terminal taxa with comments on areas of taxonomic instability and recent change. *Chelonian Research Monographs*, **4**, 173–199.

Blazej RG, Kumaresan P, Mathies RA (2008) Microfabricated bioprocessor for integrated nanoliter-scale Sanger DNA sequencing. *Proceedings of the National Academy of Sciences USA*, **103**, 7240–7245.

Bortolus A, Schwindt E (2007) What would Darwin have written now? *Biodiversity and Conservation*, **16**, 337–345.

Bradley RD, Baker RJ (2001) A test of the genetic species concept: cytochrome-b sequences and mammals. *Journal of Mammalogy*, **82**, 960–973.

Brodie EL, DeSantis TZ, Parker JPM et al. (2007) Urban aerosols harbor diverse and dynamic bacterial populations. *Proceedings of the National Academy of Sciences USA*, **104**, 299–304.

Brooks T, Tobias J, Balmford A (1999) Deforestation and bird extinctions in the Atlantic forest. *Animal Conservation*, **2**, 211–222.

Brown DM, Brenneman RA, Koepfli K-P et al. (2007) Extensive population genetic structure in the giraffe. *BMC Biology*, **5**, 57.

Buckley LB, Jetz W (2007) Environmental and historical constraints on global patterns of amphibian richness. *Proceedings of the Royal Society B*, **274**, 1167–1173.

Carr SM, Ballinger SW, Derr JN, Blankenship LH, Bickham JW (1986) Mitochondrial DNA analysis of hybridization between sympatric white-tailed and mule deer in west Texas. *Proceedings of the National Academy of Sciences USA*, **83**, 9576–9580.

Castiglioni B, Rizzi E, Frosini A et al. (2004) Development of a universal microarray based on the ligation detection reaction and 16S rRNA gene polymorphism to target diversity of cyanobacteria. *Applied Environmental Microbiology*, **70**, 7161–7172.

Cathey JC, Bickham JW, Patton JC (1998) Introgressive hybridization and nonconcordant evolutionary history of maternal and paternal lineages in North American deer. *Evolution*, **52**, 1224–1229.

Chase MW, Salamin N, Wilkinson M et al. (2005) Land plants and DNA barcodes: short-term and long-term goals. *Philosophical Transactions of the Royal Society B*, **360**, 1889–1895.

Chown SL, Sinclair BJ, Leinaas HP, Gaston KJ (2004) Hemispheric asymmetries in biodiversity – a serious matter for ecology. *PLoS Biology*, **2**, 1701–1707.

Cohan FM (2002) What are bacterial species? *Annual Review of Microbiology*, **56**, 457–487.

Colborn J, Crabtree RE, Shaklee JB, Pfeiler E, Bowen BW (2001) The evolutionary enigma of bonefishes (Albula spp.): cryptic species and ancient separations in a globally distributed shorefish. *Evolution*, **55**, 807–820.

Cook BD, Page TJ, Hughes JM (2008) Importance of cryptic species for identifying 'representative' units of biodiversity for freshwater conservation. *Biological Conservation*, **141**, 2821–2831.

Cottrell MT, Waidner LA, Yu L, Kirchman DL (2005) Bacterial diversity of metagenomic and PCR libraries from the Delaware River. *Environmental Microbiology*, **7**, 1883–1895.

Cox N, Chanson J, Stuart S (2006) *The Status and Distribution of Reptiles and Amphibians of the Mediterranean Basin*. The World Conservation Union (IUCN), Gland, Switzerland, and Cambridge, UK.

Cracraft J (2002) The seven great questions of systematic biology: an essential foundation for conservation and the sustainable use of biodiversity. *Annals of the Missouri Botanical Garden*, **89**, 127–144.

Cracraft J, Feinstein J, Vaughn J, Helm-Bychowski K (1998) Sorting out tigers (*Panthera tigris*): mitochondrial sequences, nuclear inserts, systematics, and conservation genetics. *Animal Conservation*, **1**, 139–150.

Curtis TP, Sloan WT (2004) Prokaryotic diversity and its limits: microbial community structure in nature and implications for microbial ecology. *Current Opinion in Microbiology*, **7**, 221–226.

Darwin CR (1845) *The Voyage of the Beagle*, 2nd edn. John Murray, London.

Darwin CR (1859) *On the Origin of Species by Means of Natural Selection, or the Preservation of Favored Races in the Struggle for Life*. John Murray, London.

DeSalle R, Amato G (2004) The expansion of conservation genetics. *Nature Reviews*, **5**, 702–712.

DeSalle R, Egan MG, Siddall M (2005) The unholy trinity: taxonomy, species delimitation and DNA barcoding. *Philosophical Transactions of the Royal Society B*, **360**, 1905–1916.

de Queiroz K (2005) Ernst Mayr and the modern concept of species. *Proceedings of the National Academy of Sciences USA*, **102**, 6600–6607.

Drmanac S, Kita D, Labat I et al. (1998) Accurate sequencing by hybridization for DNA diagnostics and individual genomes. *Nature Biotechnology*, **16**, 54–58.

Dubois A (2003) The relationships between taxonomy and conservation biology in the century of extinctions. *Comptes Rendus Biologies*, **326**, S9–S21.

Dudgeon D, Arthington AH, Gessner MO et al. (2006) Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Review*, **81**, 163–182.

Dyer LA, Singer MS, Lill JT et al. (2007) Host specificity of Lepidoptera in tropical and temperate forests. *Nature*, **448**, 696–700.

Dykhuizen DE (1998) Santa Rosalia revisited: why are there so many species of bacteria? *Antonie van Leeuwenhoek*, **73**, 25–33.

Ekrem T, Willassen E, Stur E (2007) A comprehensive DNA sequence library is essential for identification with DNA barcodes. *Molecular Phylogenetics and Evolution*, **43**, 530–542.

Ellison CK, Burton RS (2005) Application of bead array technology to community dynamics of marine phytoplankton. *Marine Ecology Progress Series*, **288**, 75–85.

Elmer KR, Dávila JA, Lougheed SC (2007) Cryptic diversity and deep divergence in an upper Amazonian leaflitter frog, *Eleutherodactylus ockendeni*. *BMC Evolutionary Biology*, **7**, 247.

Embley TM, Stackebrandt E (1997) Species in practice: explaining uncultured prokaryotic diversity in natural samples. In: *Species: The Units of Biodiversity* (eds. Claridge MF, Dawah HA, Wilson MR), pp. 61–81. Chapman & Hall, London.

Erwin TL (1982) Tropical forests: their richness in Coleoptera and other arthropod species. *Coleoptera Bulletin*, **36**, 74–75.

Faith DP (1992) Conservation evaluation and phylogenetic diversity. *Biological Conservation*, **61**, 1–10.

Fernandez CC, Shevock JR, Glazer AN, Thompson JN (2006) Cryptic species within the cosmopolitan desiccation-tolerant moss *Grimmia laevigata*. *Proceedings of the National Academy of Sciences USA*, **103**, 637–642.

Fouquet A, Gilles A, Vences M et al. (2007) Underestimation of species richness in neotropical frogs revealed by mtDNA analyses. *PLoS ONE*, **10**, e1109.

Frias-Lopez J, Shi Y, Tyson GW et al. (2008) Microbial community gene expression in ocean surface waters. *Proceedings of the National Academy of Sciences USA*, **105**, 3805–3810.

Fritts TH, Rodda GH (1998) The role of introduced species in the degradation of island ecosystems: a case history of Guam. *Annual Review of Ecology and Systematics*, **29**, 113–140.

Frost DR (2006) *Amphibian Species of the World, an Online Reference* (American Museum of Natural History, New York), Version 4. Available at http://research.amnh.org/herpetology/amphibia/index.php.

Frost JS, Bagnara JT (1977) An analysis of reproductive isolation between *Rana magnaocularis* and *Rana berlandieri forreri* (*Rana pipiens* complex). *Journal of Experimental Zoology* **202**, 291–306.

Frost JS, Platz JE (1983) Comparative assessment of modes of reproductive isolation among four species of leopard frogs (*Rana pipiens* complex). *Evolution*, **37**, 66–78.

Gao Z, Tseng C, Pei Z, Blaser MJ (2007) Molecular analysis of human forearm superficial skin bacterial biota. *Proceedings of the National Academy of Sciences USA*, **104**, 2927–2932.

Gaston KJ (1996) Biodiversity – latitudinal gradients. *Progress in Physical Geography*, **20**, 466–476.

Gaston KJ (2000) Global patterns in biodiversity. *Nature*, **405**, 220–227.

Gevers D, Cohan FM, Lawrence JG et al. (2005) Re-evaluating prokaryotic species. *Nature Reviews in Microbiology*, **3**, 733–739.

Gibbons JW, Scott DE, Ryan TJ et al. (2000) The global decline of reptiles, déjà vu amphibians. *BioScience*, **50**, 653–666.

Glaw F, Köhler J (1998) Amphibian species diversity exceeds that of mammals. *Herpetological Review*, **29**, 11–12.

Glowka L, Burhenne-Guilmin F, Synge H (1994) *A Guide to the Convention on Biological Diversity*. International Union for Conservation of Nature and Natural Resources (IUCN), Gland, Switzerland, and Cambridge, UK.

Gonder MK, Disotell TR, Oates JF (2006) New genetic evidence on the evolution of chimpanzee populations and implications for taxonomy. *International Journal of Primatology*, **27**, 1103–1127.

Goodfellow M, Manfio GP, Chun J (1997) Towards a practical species concept for cultivable bacteria. In: *Species: The Units of Biodiversity* (eds. Claridge MF, Dawah HA, Wilson MR), pp. 25–60. Chapman & Hall, London.

Govind VK, Hoang VN, Stickel A et al. (2003) An inexpensive and portable microchip-based platform for integrated RT-PCR and capillary electrophoresis. *Analyst*, **133**, 331–338.
Green BD, Keller M (2006) Capturing the uncultivated majority. *Current Opinion in Biotechnology*, **17**, 236–240.
Grenyer R, Orme CDL, Jackson SF et al. (2006) Global distribution and conservation of rare and threatened vertebrates. *Nature*, **444**, 93–96.
Hajibabaei M, Singer GAC, Hebert PDN, Hickey DA (2007) DNA barcoding: how it complements taxonomy, molecular phylogenetics, and population genetics. *Trends in Genetics*, **23**, 167–172.
Hammond PM (1992) Species inventory. In: *Global Diversity: Status of the Earth's Living Resources* (ed. Groombridge B), pp. 17–39. Chapman & Hall, London.
Hart MW, Keever CC, Dartnall AJ, Byrne M (2006) Morphological and genetic variation indicate cryptic species within Lamarck's little sea star, *Parvulastra (=Patiriella) exigua*. *Biological Bulletin*, **210**, 158–167.
Harvey AL (2008) Natural products in drug discovery. *Drug Discovery Today*, **13**, 894–901.
Hawkins BA (2001) Ecology's oldest pattern? *Trends in Ecology & Evolution*, **16**, 470.
Hebert PDN, Cywinska A, Ball SL, De Waard JR (2003a) Biological identification through DNA barcodes. *Proceedings of the Royal Society London B*, **270**, 313–321.
Hebert PDN, Ratnasingham S, De Waard JR (2003b) Barcoding animal life: cytochrome c oxidase subunit I divergences among closely related species. *Proceedings of the Royal Society London B*, **270**, S96–S99.
Hebert PDN, Penton EH, Burns JM, Janzen DH, Hallwachs W (2004) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly Astraptes fulgerator. *Proceedings of the National Academy of Sciences USA*, **101**, 14812–14817.
Hey J (2001) The mind of the species problem. *Trends in Ecology & Evolution*, **16**, 326–329.
Hillis DM (1981) Premating isolating mechanisms among three species of the *Rana pipiens* complex in Texas and southern Oklahoma. *Copeia*, 1981, 312–319.
Hillis DM (1988) Systematics of the Rana pipiens complex: puzzle and paradigm. *Annual Review of Ecology and Systematics*, **19**, 39–63.
Hillis DM, Frost JS, Wright D (1983) Phylogeny and biogeography of the *Rana pipiens* complex: a biochemical evaluation. *Systematic Zoology*, **32**, 132–143.
Hillis DM, Wilcox TS (2005) Phylogeny of the New World true frogs (*Rana*). *Molecular Phylogenetics and Evolution*, **34**, 299–314.
Horner-Devine MC, Lage M, Hughes JB, Bohaman BJM (2004) A taxa-area relationship for bacteria. *Nature*, **432**, 750–753.
Huber JA, Mark Welch DB, Morrison HG et al. (2007) Microbial population structures in the deep marine biosphere. *Science*, **318**, 97–100.
Huse SM, Dethlefsen L, Huber JA et al. (2008) Exploring microbial diversity and taxonomy using SSU rRNA hypervariable tag sequencing. *PLoS Genetics*, **4**, 1–10.
Isaac NJB, Turvey ST, Collen B, Waterman C, Baillie JEM (2007) Mammals on the EDGE: conservation priorities based on threat and phylogeny. *PLoS ONE*, **3**, e296.
IUCN Red List of Threatened Species™ (2008) *State of the World's Species*. Available at http://www.iucn.org/redlist.
IWC (2007) Report of the Scientific Committee. *Journal of Cetacean Research and Management*, 10 (Suppl.).
Johnson TH, Stattersfield AJ (2008) A global review of island endemic birds. *Ibis*, **132**, 167–180.
Köhler J, Vieites DR, Bonett RM et al. (2005) New amphibians and global conservation: a boost in species discoveries in a highly endangered vertebrate group. *BioScience*, **55**, 693–696.
Lim BK, Engstrom, MD, Bickham JW, Patton JC (2008) Molecular phylogeny of New World sheath-tailed bats (*Emballonuridae: Diclidurini*) based on loci from the four genetic transmission systems in mammals. *Biological Journal of the Linnean Society*, **93**, 189–209.
Linder HP (1995) Setting conservation priorities: the importance of endemism and phylogeny in the southern African orchid genus Herschelia. *Conservation Biology*, **9**, 585–595.
Littlejohn MJ, Oldham RS (1968) *Rana pipiens* complex: mating call structure and taxonomy. *Science*, **162**, 1003–1005.

Lundberg JG, Kottelat M, Smith GR, Stiassny MLJ, Gill AC (2000) So many fishes, so little time: an overview of recent ichthyological discovery in continental waters. *Annals of the Missouri Botanical Garden*, **87**, 26–62.

MacKinnon J (2000) New mammals in the 21st century? *Annals of the Missouri Botanical Garden*, **87**, 63–66.

Marsh TL, Saxman P, Cole J, Tiedje J (2000) Terminal restriction fragment length polymorphism analysis program, a web-based research tool for microbial community analysis. *Applied and Environmental Microbiology*, **66**, 3616–3620.

May RM (1988) How many species are there on Earth? *Science*, **241**, 1441–1449.

May RM (1990) How many species? *Philosophical Transactions Royal Society London B*, **330**, 293–304.

May RM (1992) How many species inhabit the Earth? *Scientific American* (October), 18–24.

May RM (1998) The dimensions of life on Earth. In: *Nature and Human Society: The Quest for a Sustainable World* (ed. Raven PH), pp. 30–45. National Academy Press, Washington, DC.

May RM, Lawton JH, Stork NE (1995) Assessing extinction rates. In: *Extinction Rates* (eds. Lawton JH, May RM), pp. 1–25. Oxford University Press, Oxford.

Mayden RL (1997) A hierarchy of species concepts: the denouement in the saga of the species problem. In *Species: The Units of Biodiversity* (eds. Claridge MF et al.), pp. 381–424, Chapman & Hall, London.

Mayr E (1946) The number of species of birds. *The Auk*, **63**, 64–69.

Mayr E (1969) *Principles of Systematic Zoology*. McGraw-Hill, Inc., New York.

Mecham JS (1971) Vocalizations of the leopard frog, *Rana pipiens*, and three related Mexican species. *Copeia*, 1971, 505–516.

Mittermeier RA, Myers N, Thomsen JB, da Fonseca GAB, Olivieri S (1998) Biodiversity hotspots and major tropical wilderness areas: approaches to setting conservation priorities. *Conservation Biology*, **12**, 516–520.

Monroe BL Jr, Sibley CG (1990) *Distribution and Taxonomy of Birds of the World*. Yale University Press, New Haven, CT.

Moore JA (1944) Geographic variation in *Rana pipiens Schreber* of eastern North America. *Bulletin of the American Museum of Natural History*, **82**, 345–370.

Moore JA (1975) *Rana pipiens* – the changing paradigm. *American Zoologist*, **15**, 837–849.

Moritz C (1994) Defining 'evolutionary significant units' for conservation. *Trends in Ecology and Evolution*, **9**, 373–375.

Munch K, Boomsma W, Willerslev E, Nielsen R (2008) Fast phylogenetic DNA barcoding. *Philosophical Transactions of the Royal Society B*, **263**, 3997–4002.

Myers N (1988) Threatened biotas: "hotspots" in tropical forests. *Environmentalist*, **8**, 187–208.

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. *Nature*, **403**, 853–858.

Nee S, May RM (1997) Extinction and the loss of evolutionary history. *Science*, **278**, 692–694.

Neufeld JD, Yu Z, Lam W, Mohn WW (2004) Serial analysis of ribosomal sequence tags (SARST): a high-throughput method for profiling complex microbial communities. *Environmental Microbiology*, **6**, 131–144.

Nilsson G (2005) *Endangered Species Handbook*. Animal Welfare Institute, Washington, DC.

Novotny V, Miller SE, Hulcr J et al. (2007) Low beta diversity of herbivorous insects in tropical forests. *Nature*, **448**, 692–697.

Ødegaard F (2000) How many species of arthropods? Erwin's estimate revised. *Biological Journal of the Linnean Society*, **71**, 583–597.

Pace NR (1997) A molecular view of microbial diversity and the biosphere. *Science*, **276**, 734–740.

Pace NR, Stahl DA, Lane DL, Olsen GJ (1986) The analysis of natural microbial populations by rRNA sequences. *Advances in Microbial Ecology*, **9**, 1–55.

Paetkau D (1999) Using genetics to identify intraspecific conservation units: a critique of current methods. *Conservation Biology*, **13**, 1507–1509.

Patterson BD (2000) Patterns and trends in the discovery of new neotropical mammals. *Diversity and Distributions*, **6**, 145–151.

Patterson BD (2001) Fathoming tropical biodiversity: the continuing discovery of neotropical mammals. *Diversity and Distributions*, **7**, 191–196.
Peterson AT (1998) New species and new species limits in birds. *The Auk*, **115**, 555–558.
Pfenninger M, Schwenk K (2007) Cryptic animal species are homogeneously distributed among taxa and biogeographical regions. *BMC Evolutionary Biology*, **7**, 121.
Pimentel D, Wilson C, McCullum C et al. (1997) Economic and environmental benefits of biodiversity. *BioScience*, **47**, 747–757.
Pimm SL, Russell GJ, Gittleman JL, Brooks TM (1995) The future of biodiversity. *Science*, **269**, 347–350.
Raup DM (1986) Biological extinction in Earth history. *Science*, **231**, 1528–1533.
Raup DM (1994) The role of extinction in evolution. *Proceedings of the National Academy of Sciences USA*, **91**, 6758–6763.
Reid WV (1998) Biodiversity hotspots. *Trends in Ecology & Evolution*, **13**, 275–280.
Ribon R, Simon JE, de Mattos GT (2003) Bird extinctions in Atlantic forest fragments of the Viçosa region, southeastern Brazil. *Conservation Biology*, **17**, 1827–1839.
Ricciardi A, Rasmussen JB (1999) Extinction rates of North American freshwater fauna. *Conservation Biology*, **13**, 1220–1222.
Ricklefs RE (2004) A comprehensive framework for global patterns in biodiversity. *Ecology Letters*, **7**, 1–15.
Roca AL, Georgiadis N, Pecon-Slattery J, O'Brien SJ (2001) Genetic evidence for two species of elephant in Africa. *Science*, **293**, 1473–1477.
Rosenzweig ML (1992) Species diversity gradients: we know more and less than we thought. *Journal of Mammalogy*, **73**, 715–730.
Rosenzweig ML (1995) *Species Diversity in Space and Time*. Cambridge University Press, Cambridge, UK.
Ryder OA (1986) Species conservation and systematics: the dilemma of subspecies. *Trends in Ecology and Evolution*, **1**, 9–10.
Savolainen V, Cowan RS, Vogler AP, Roderick GK, Lane R (2005) Towards writing the encyclopedia of life: an introduction to DNA barcoding. *Philosophical Transactions of the Royal Society B*, **360**, 1805–1811.
Schipper J, Chanson JS, Chiozza F et al. (2008) The status of the world's land and marine mammals: diversity, threat, and knowledge. *Science*, **322**, 225–230.
Schloss PD, Handelsman J (2004) Status of the microbial census. *Microbiology and Molecular Biology Reviews*, **68**, 686–691.
Schloss PD, Handelsman J (2005) Metagenomics for studying unculturable microorganisms: cutting the Gordian knot. *Genome Biology*, **6**, 229.
Schloss PD, Handelsman J (2006) Toward a census of bacteria in soil. *PLoS Computational Biology*, **2**, e92.
Schütte UME, Abdo Z, Bent SJ et al. (2008) Advances in the use of terminal restriction fragment length polymorphism (T-RFLP) analysis of 16S rRNA genes to characterize microbial communities. *Applied Microbiology and Biotechnology*, **80**, 365–380.
Sechrest W, Brooks TM, da Fonseca GAB et al. (2002) Hotspots and the conservation of evolutionary history. *Proceedings of the National Academy of Sciences USA*, **99**, 2067–2071.
Sites JW Jr, Marshall JC (2003) Delimiting species: a Renaissance issue in systematic biology. *Trends in Ecology and Evolution*, **18**, 462–470.
Smith MA, Poyarkov NA Jr, Hebert PDN (2008) CO1 DNA barcoding amphibians: take the chance, meet the challenge. *Molecular Ecology Resources*, **8**, 235–246.
Sogin ML, Morrison HG, Huber JA, Welch DM, Huse SM et al. (2006) Microbial diversity in the deep sea and the underexplored "rare biosphere." *Proceedings of the National Academy of Sciences USA*, **103**, 12115–12120.
State of Observed Species (2008) International Institute for Species Exploration, Arizona State University, http://species.asu.edu/index.
Stork NE (1993) How many species are there? *Biodiversity and Conservation*, **2**, 215–232.
Stork NE (2007) World of insects. *Nature*, **448**, 657–658.
Stuart BL, Inger RF, Voris HK (2006) High level of cryptic species diversity revealed by sympatric lineages of Southeast Asian forest frogs. *Biological Letters*, **2**, 470–474.
Stuart SN, Chanson JS, Cox NA et al. (2004) Status and trends of amphibian declines and extinctions worldwide. *Science*, **306**, 1783–1786.

Systematics Agenda 2000 (1994) *Systematics Agenda 2000: Charting the Biosphere*. New York: Society of Systematic Biologists, American Society of Plant Taxonomists, Willi Hennig Society, Association of Systematics Collections.

Trevino HS, Skibiel AL, Karels TJ, Dobson FS (2007) Threats to avifauna on oceanic islands. *Conservation Biology*, **21**, 125–132.

Tringe SG, von Mering C, Kobayashi A et al. (2005) Comparative metagenomics of microbial communities. *Science*, **308**, 554–557.

Trujillo RG, Patton JC, Schlitter DA, Bickham JW (2009) Molecular phylogenetics of the bat genus *Scotophilus (Chiroptera: Vespertilionidae)*: perspectives from paternally and maternally inherited genomes. *Journal of Mammalogy* **90**, 548–560.

Turvey ST, Pitman RL, Taylor BL et al. (2007) First human-caused extinction of a cetacean species? *Biological Letters*, **3**, 537–540.

Urbina-Cardona JN (2008) Conservation of neotropical herpetofauna: research trends and challenges. *Tropical Conservation Sciences*, **1**, 359–375.

Vane-Wright RI, Humphries CJ, Williams PH (1991) What to protect? – Systematics and the agony of choice. *Biological Conservation*, **55**, 235–254.

Vences M, Thomas M, Van Der Meijden A, Chiari Y, Vietes DR (2005) Comparative performance of the 16S rRNA gene in DNA barcoding of amphibians. *Frontiers in Zoology*, **2**, 5.

Venter J, Remmington K, Heidelberg J et al. (2004) Environmental genome shotgun sequencing of the Sargasso Sea. *Science*, **304**, 66–74.

Wake DB, Vredenburg VT (2008) Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proceedings of the National Academy of Sciences USA*, **105**, 11466–11473.

Wallace AR (1869) *The Malay Archipelago: The Land of the Orang-utan, and the Bird of Paradise. A Narrative of Travel, with Studies of Man and Nature*. Harper & Brothers, New York.

Ward BB (2002) How many species of prokaryotes are there? *Proceedings of the National Academy of Sciences USA*, **99**, 10234–10236.

Wayne LG, Brenner DJ, Colwell RR et al. (1987) Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *International Journal of Systematic Bacteriology*, **37**, 463–464.

Wheeler QD (2004) Taxonomic triage and the poverty of phylogeny. *Philosophical Transactions of the Royal Society London B*, **359**, 571–583.

Whitaker RJ, Grogan DW, Taylor JW (2003) Geographic barriers isolate endemic populations of hyperthermophilic Archaea. *Science*, **301**, 976–978.

Wilson DE, Reeder DM, editors (1993) *Mammal Species of the World: A Taxonomic and Geographic Reference*, 2nd edn. Smithsonian Institution Press, Washington.

Wilson DE, Reeder DM, editors (2005) *Mammal Species of the World: A Taxonomic and Geographic Reference*, 3rd edn. Johns Hopkins University Press, Baltimore, Maryland.

Wilson EO (1985) The biological diversity crisis. *BioScience*, **35**, 700–706.

Wilson, EO (2003) The encyclopedia of life. *Trends in Ecology and Evolution*, **18**, 77–80.

Wilson EO (2006) *The Creation: An Appeal to Save Life on Earth*. W. W. Norton and Co., Inc., New York.

Wilting A, Buckley-Beason VA, Feldhaar H et al. (2007) Clouded leopard phylogeny revisited: support for species recognition and population division between Borneo and Sumatra. *Frontiers in Zoology*, **4**, 15.

Woese CR (1987) Bacterial evolution. *Microbiology Review*, **51**, 221–271.

Woese CR (1994) There must be a prokaryote somewhere: microbiology's search for itself. *Microbiological Reviews*, **58**, 1–9.

# Index