Biodiversitas – ICB Mataram Lombok 2019

Evolutionary history & global spread of termites

a mitochondrial phylogenomics approach

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Epifamily Isoptera / Order Blattodea

Termites – ‘same wing’

A small group, by insect standards

About 3000 described species

But evolutionary relationships poorly understood

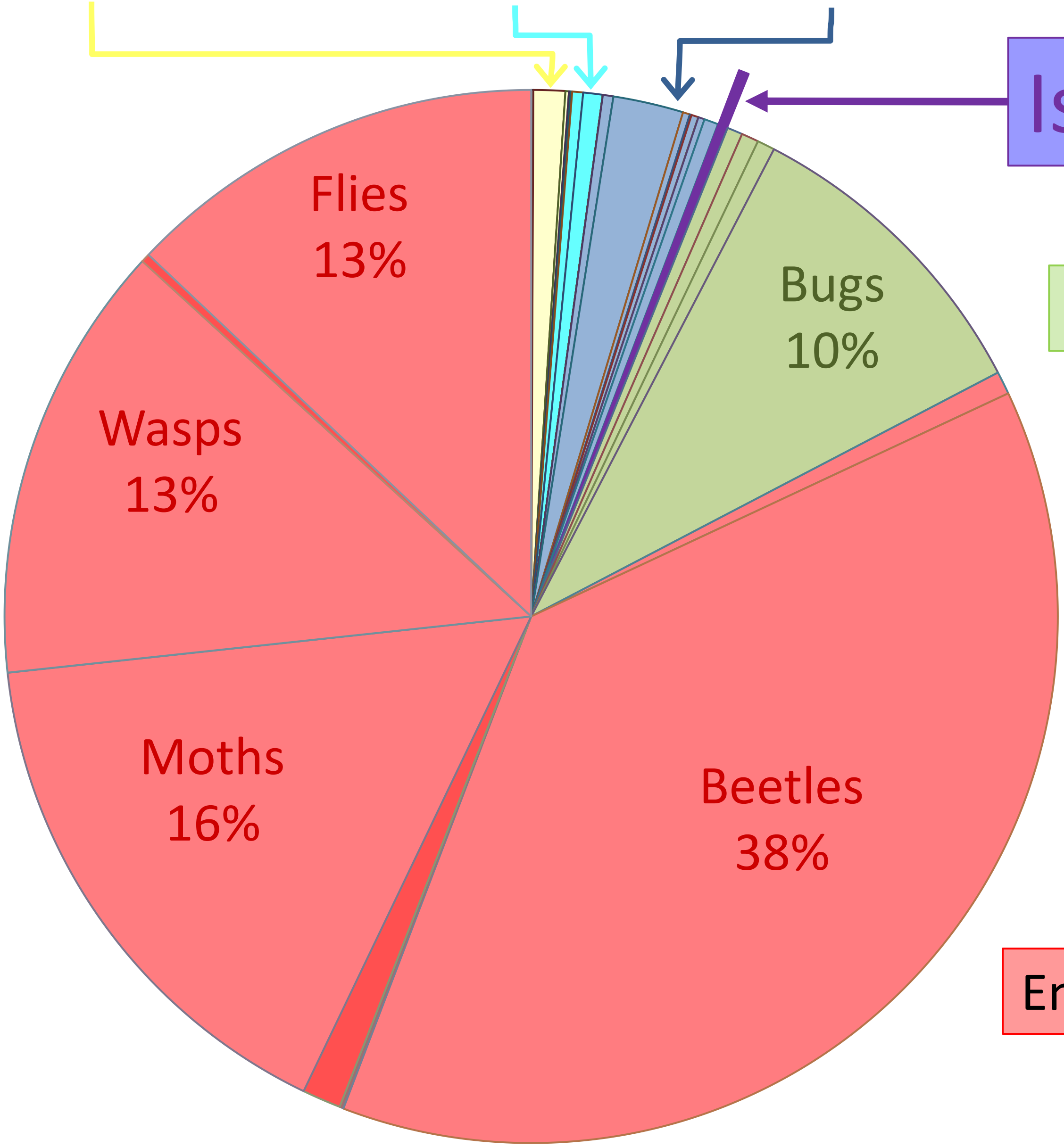
Photos by Jan Šobotník, Quah

Apterous

Palaeoptera

Polyneoptera

Isoptera 0.3%



Paraneoptera

Endopterygota

Economically important

- The major urban pest insect in $$
- 50% of urban pest control market
- AUD 1 bn pa Australia;
  10,500 employees
- USD 22 bn pa globally
- Destroy electrical cabling
- Major forestry pests
  - Various worldwide
- Agricultural pests
  - Various worldwide
  - not in Australia

J Creffield

Courir Mail

JNK Maniania

Ecologically important

- Termites comprise 10-20% of animal biomass in the tropics
- Major decomposers, 10-50% of the dead plant matter
- Increase soil carbon
- Increase soil nitrogen, with symbiotic gut bacteria that fix N_2,
  - similar scale as legumes?

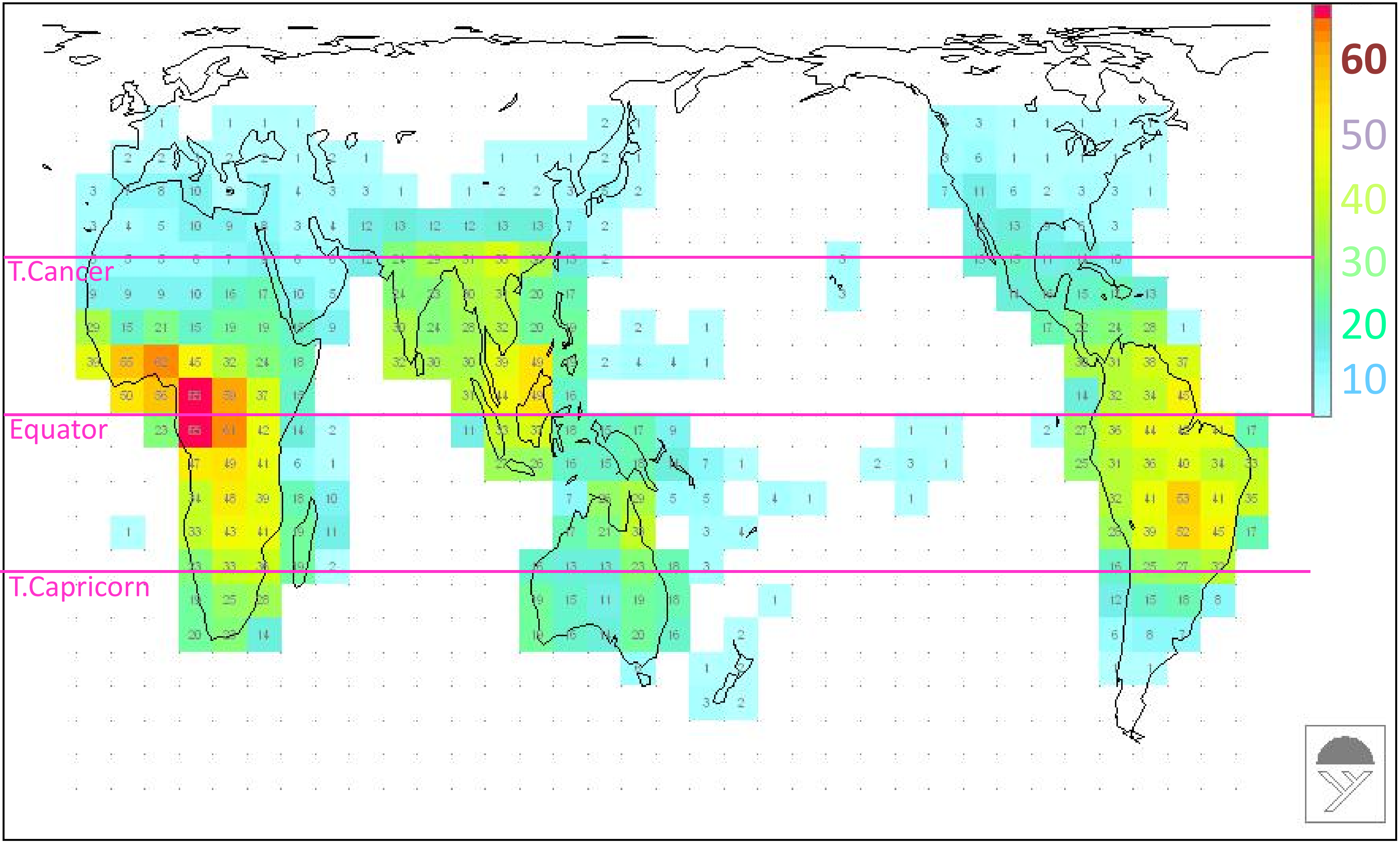
- Increase water infiltration, soil moisture
- Major soil turnover, < 1 T/ha/yr

- Major food source

P Gleeson

Termite generic diversity

Red (max) = 65

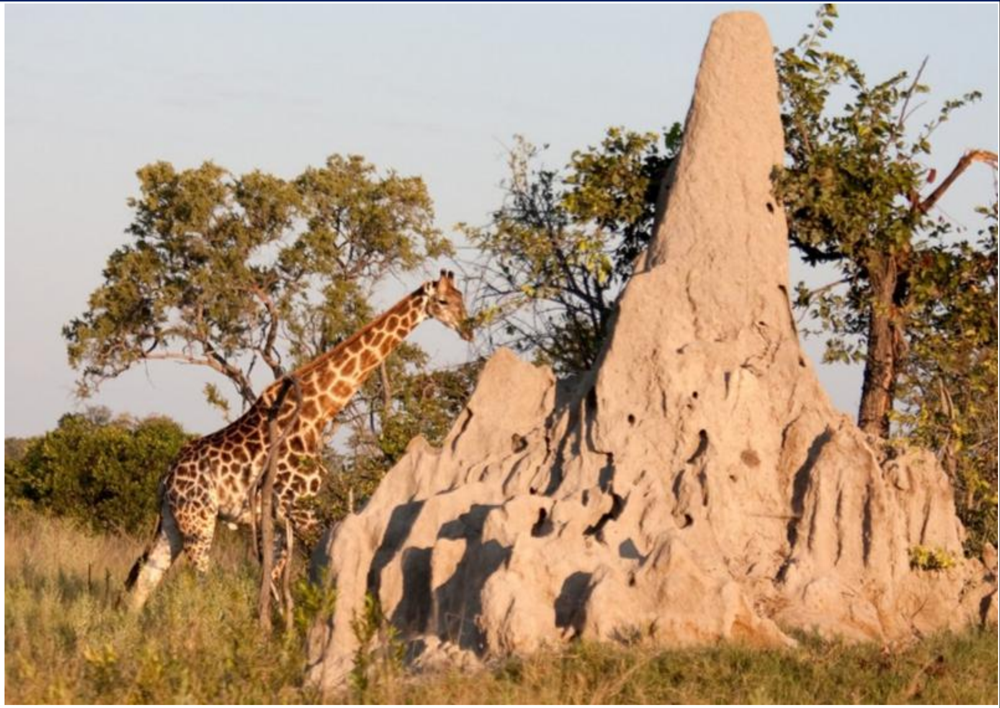


Paul Eggleton, Natural History Museum, London

Global patterns of termite diversity

Pattern #1

Termites like it hot!
Highest diversity in tropics
None beyond 45° N & S



Pattern #2

Continental differences in termite diversity: consider tropics only

Africa >> South America > Asia > Australia

Some of the difference is land area:
more land = more diversity

Most due to other evolutionary factors

Phylogeny uncertain

Mixture of primitive and advanced features in:

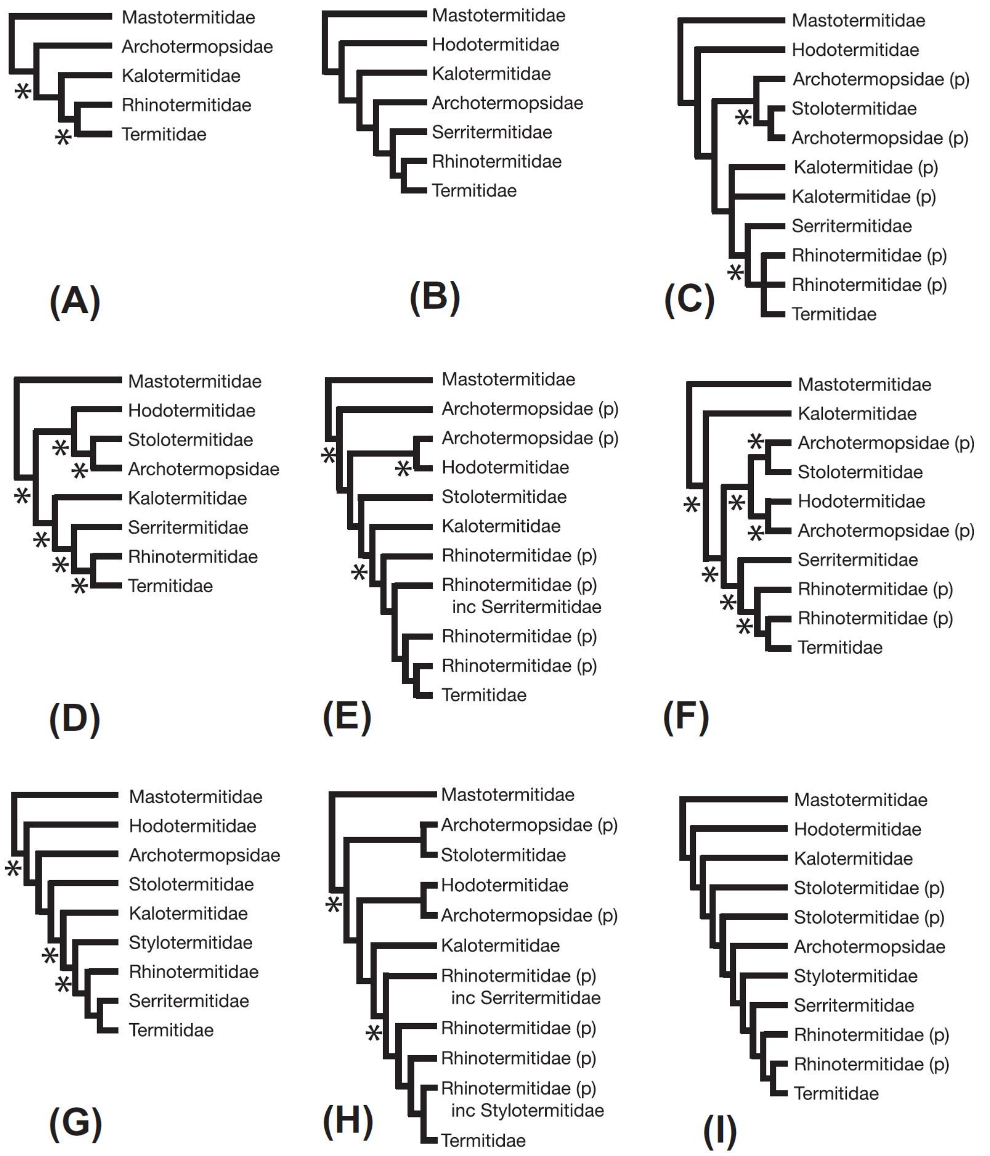
- anatomy
- defensive adaptions
- social behaviours
- feeding / digestion

Best study:

- 3 fragments = 2,000 bp DNA
- 230 spp

Most studies

- 2-3 fragment = 500–1,000 bp DNA
- 20-40 species



(A) Kambhampati et al 1996, (B) Kambhampati & Eggleton 2000, (C) Donovan et al 2000, (D) Thompson et al 2000, (E) Inward et al 2007, (F) Legendre et al 2008, (G) Engel et al 2009, (H) Ware et al 2010, (I) Lo & Eggleton 2011

‘Lower termites’ – basal confusion

With gut flagellate protozoa. Which most basal?

Mastotermes **–**
Giant termite ‘living fossil’
most primitive anatomy,
but ‘advanced’ social behaviour?

Termopsidae –
‘dampwood’ termites
Kalotermitidae –
‘drywood’ termites
Somewhat primitive anatomy,
but simple social behaviour

Rhinotermitidae –
‘subterranean’ termites
many wood–eating pests
1 or 2 soldier castes

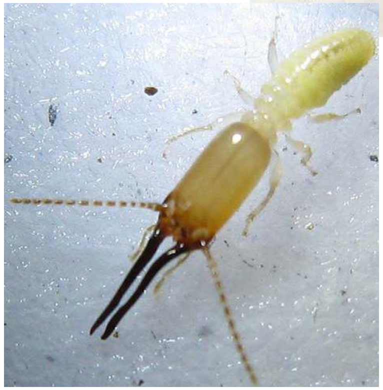
Photos by Jan Šobotník, Quah

‘Higher termites’ – confusion up top

Termitidae – no gut flagellate protozoa

Macrotermitinae –
‘advanced’ fungus growers
Biter soldiers

Termitinae –
diverse, incl. wood,
grass, soil feeding,
biters/snappers,
toxic soldiers

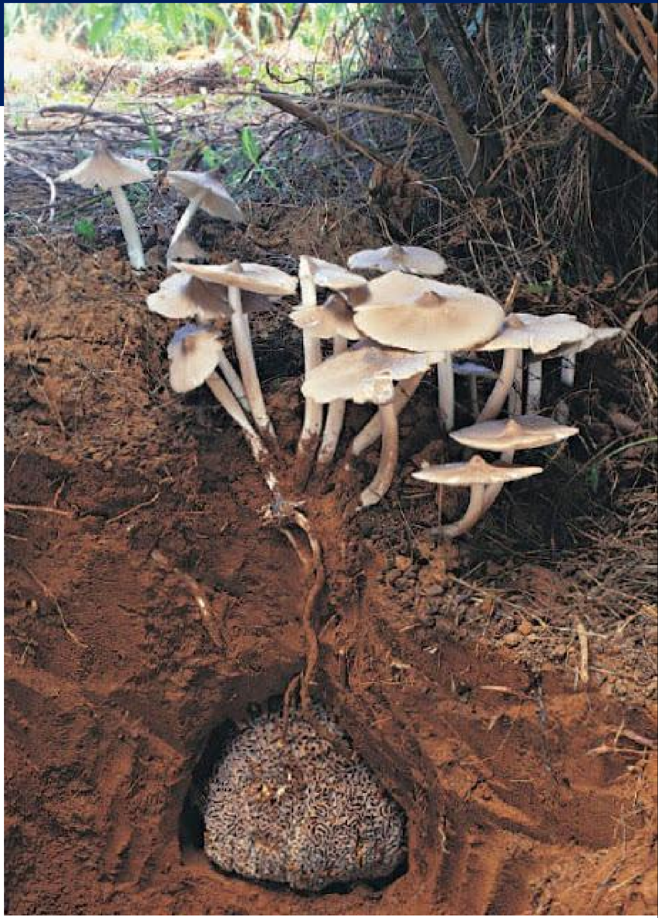


Nasutitermitinae –
diverse, incl. wood,
grass, soil feeding,
all glue gun soldiers

Photos by Jan Šobotník, Quah

Most advanced?

Fungus growing seen as most derived behaviour



Two solider castes
in most species

Huge colonies (≤5M popn),
enormous queens

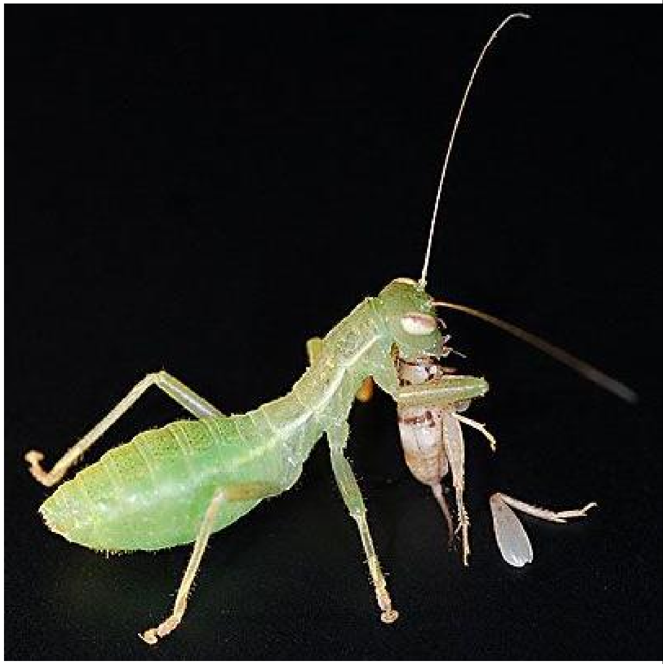
Dominant group in
African & Asian ecosystems

Phylogeny solution – mitogenomes

Mitochondrial genomes used to resolve deep relationships

2006 - used to validate Mantophasmadea (heelwalkers)

2007 + used to resolve deep, intra-ordinal relationships in flies, bugs, wasps, beetles, & moths



Splits dating from 50 to 200 MYA

Becoming almost affordable in 2011…

Then affordable by 2013

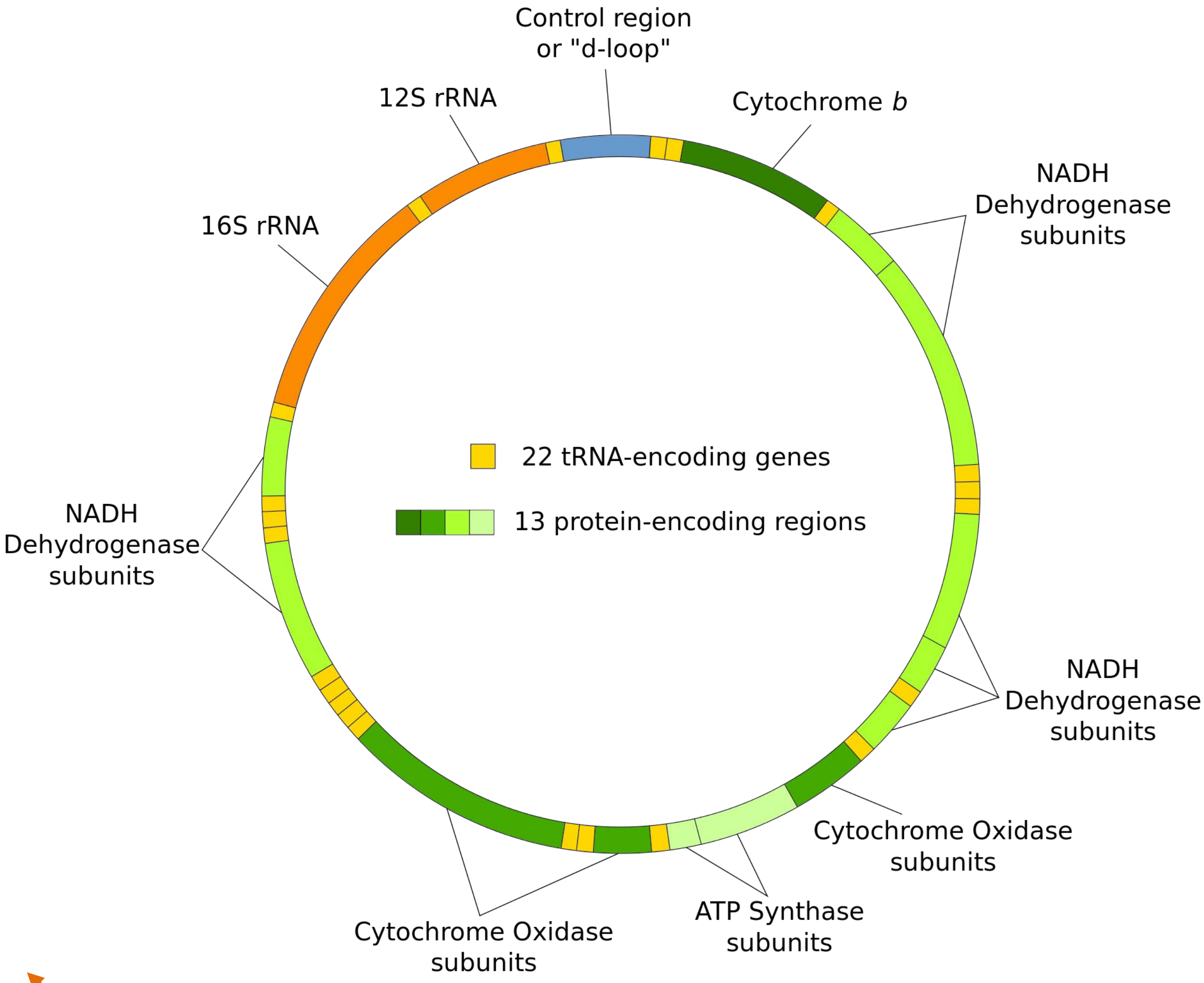
Then almost cheap by 2015!

Cameron et al (2006) *Mol. Phylogenet. Evol*. **38**, 274–279

Mt genomes

Typical metazoan mt genome is:

- Circular molecule
- ca. 15,000 bp in size
- Coding for 37 genes
  - 13 protein-coding genes (PCGs)
  - 2 ribosomal RNAs
  - 22 transfer RNAs
- PCR amplification using <u>universally conserved</u> and/or purpose designed primers



Good for deep / old relationships

Shanel Kalicharan / Wikipedia

Termite mt genome team

Mostly Australian team

Started with Stephen
(master of insect mt genomes)
when both at CSIRO

Long term collaboration with Nate
(gut flora, pop gen)

Expanded with Thomas
Postdoc at NUS, then USydney

Collaborated with Simon
(dating methods)

Stephen Cameron
CSIRO/
Purdue Univ

Thomas Bourguignon
Nat Univ Sing/
Okinawa Inst S&T

Nathan Lo
Simon Ho
Univ
Sydney

Study 1: 2011-2012

First step, resolve interfamilial relationships and define major clades

- Sequenced mt genomes of 13 termite species from all families
- Added 5 mt genomes of 4 cockroach species & 1 mantid species
- Plus 3 mt genomes of outgroups: heelwalker, phasmid & grasshopper

Almost all done manually (exception of the sequencing)!

Cameron et al (2012) *Mol Phylogen Evol* **65**, 163–173

The cookery

- DNA extracted from the head and thorax of workers with DNeasy kits
- Primers were universal insect, consensus dictyopteran, or designed specifically
- Multiple long PCR amplification with Elongase
- Sanger sequencing with ABI BigDye v3 dye terminator, on an ABI 3770 or ABI 3740 capillary sequencer
- Raw sequence files edited & assembled into contigs in Sequencher v4/5
- Transfer RNA inference was conducted using tRNAscan-SE
- Annotations of the ribosomal RNA genes were done by eye
- Annotation identified structures such as stem-loops and repeat regions
- Alignments each gene separately with Muscle implemented in MEGA5
- PCGs were aligned as DNA codons in MEGA5
- RNA genes were directly aligned as DNA
- Individual gene alignments were concatenated in MacClade 4.06

Phylogenic analyses

- **Likelihood** performed with the RaxML Black-Box webserver
- **Bayesian** inference with MrBayes ver 3.1.2

- Convergence was achieved by all analyses within 3 million generations with sampling every 1000 generations using Tracer v 1.4
- Conflict between codon- and gene-based signals was assessed by partitioning either by codon or gene for each inference method
- Base compositional bias examined using partitioning by codon, LogDet transforms in PAUP 4.0b10, PHASE, & without 3rd codons (most biased partition)
- 16 total partitions, 13 PCGs, two rRNAs and a combined partition for the 22 tRNAs as each individual tRNA is too short (ca. 65 bp)
- Partition models were chosen using AIC as implemented in ModelTest

N Gordon / Getty

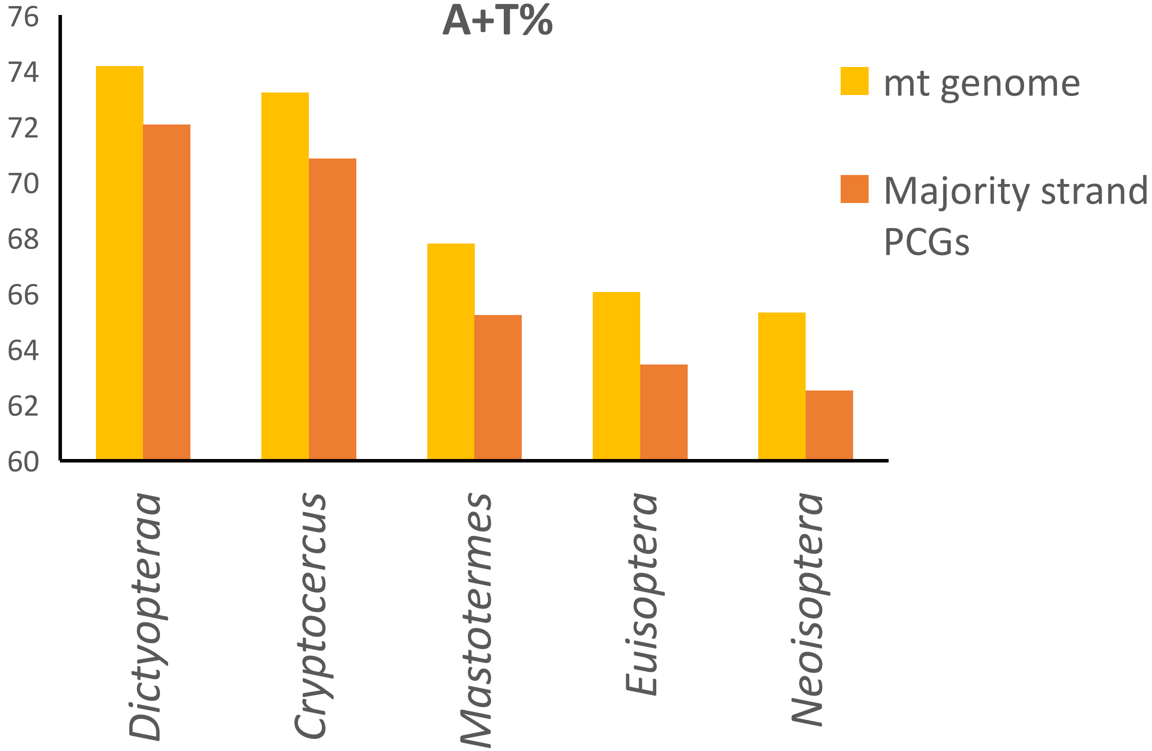
Some variation in bases

Termites have mt genomes of similar size to other metazoans
But a little larger than their cockroach ancesters/cousins

Termites 15,483 – 16,524
Cockroaches 14,996 – 15,553

Most genome size variation due to large repeat units in the A+T rich putative control regions
Largest decrease in 3rd codon

PCG = protein coding gene

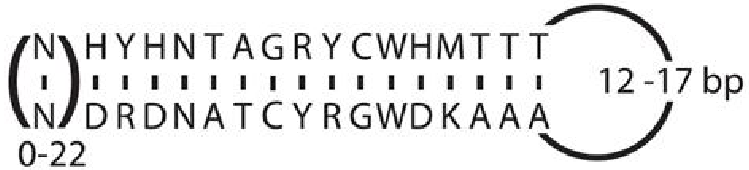


Cameron et al (2012) *Mol Phylogen Evol* **65**, 163–173

Rare genomic changes

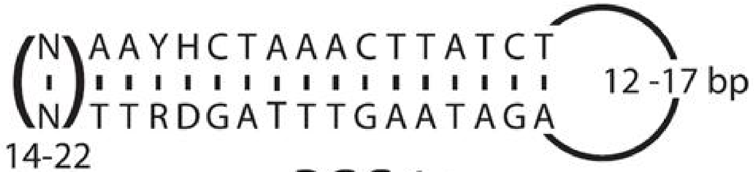
Two main RGCs:

RGC 1 is a hairpin loop in A + T rich region elongated in all termites, *Cryptocercus* and *Eupolyphaga*



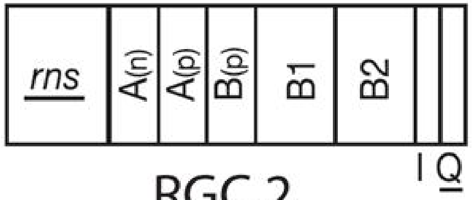
RGC 1

RGC 1a is variant with base substitutions in more conserved distal stem



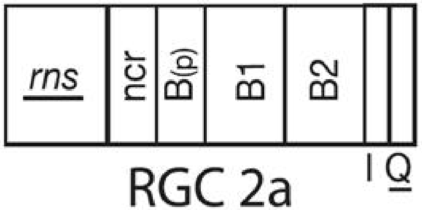
RGC 1a

RGC 2 has complicated double repeat units, type-A (186 bp) repeats at rnS end and type-B (552 bp) repeats containing hairpin loop structure at the trnI end



RGC 2

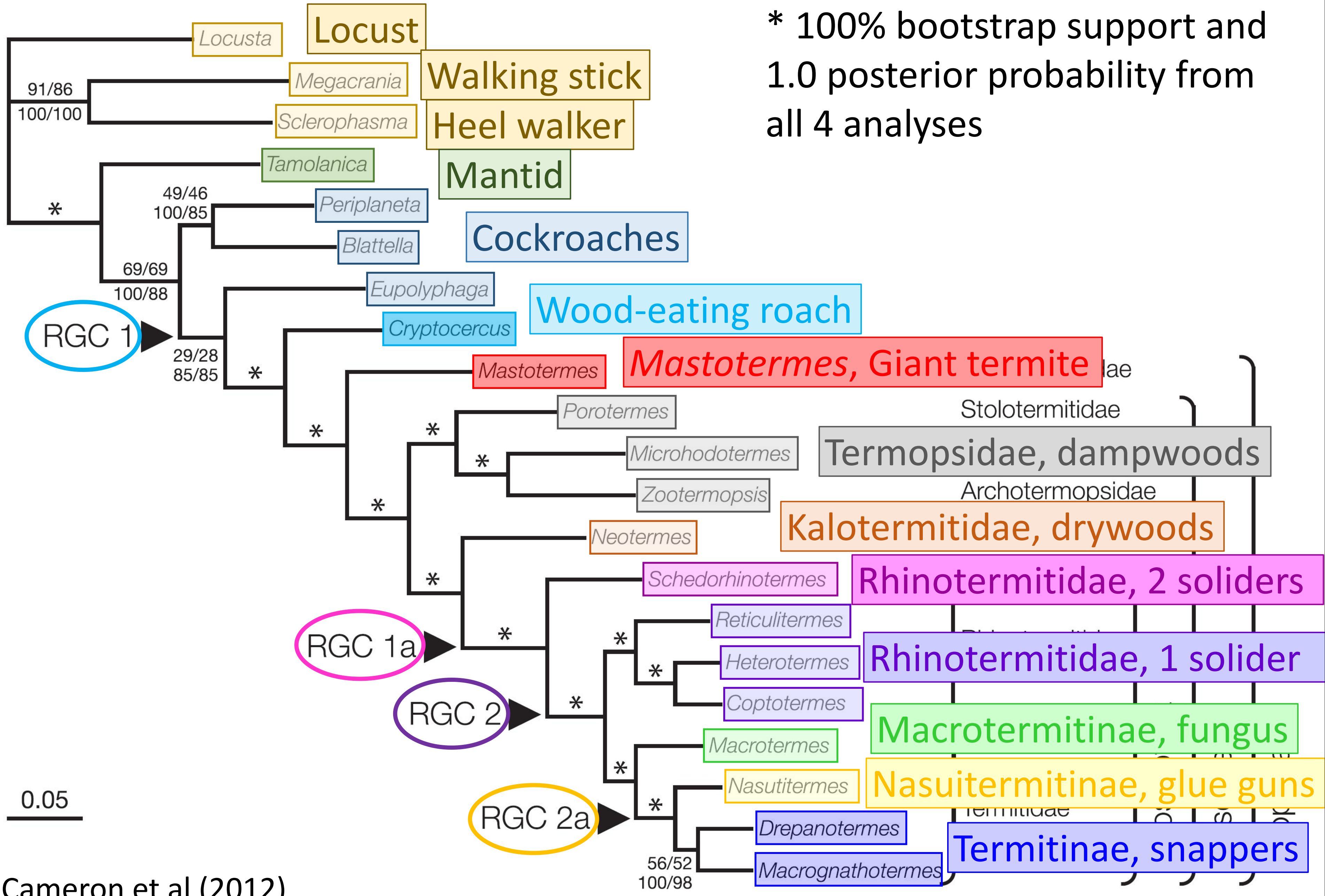
RCG 2a has lost type-A repeats



RGC 2a

Cameron et al (2012) *Mol Phylogen Evol* **65**, 163–173

* 100% bootstrap support and 1.0 posterior probability from all 4 analyses



Cameron et al (2012)

Study 2: 2012-2014

Second step, estimate the dates of the major evolutionary events

- Sequenced mt genomes of 48 new termite species
- Included 18 previous termite species
- Added 5 mt genomes of 4 cockroach species & 1 mantid species
- Plus 3 mt genomes of outgroups: heelwalker, phasmid & grasshopper

Some done manually, more automation

G Steinmetz

www.wildwebafrica.com © Wim Vorster

N Gordon / Getty

Bourguignon et al (2014) *Mol Biol Evol* **32**, 406–421

The cookery mk 2

- DNA extracted from the head of workers with TaKaRa DNAkits
- 2 x long PCR amplification with TaKaRa LA Taq
- Mixed fragments in equimolar concentration, multiplexed using Nextera XT DNA preparation kit
- Paired-end sequenced with Illumina HiSeq2000
- Sequenced the multiplexed libraries and produced ca. 31 million paired-end reads at a read length of 100 bp for the 48 samples
- Assembled sequences using the CLC suite of programs
- Removed control regions as generally poorly assembled
- Individual gene alignments were concatenated in Mesquite

TGMC

Lee Dalton / Arkive

Phylogenic analyses mk 2

- **Likelihood** performed with the RAxML Black-Box webserver
- **Bayesian** inference with MrBayes ver 3.2
- Convergence was achieved by all analyses by 2 million generations with sampling every 1000 generations using Tracer v 1.5
- Partition models were chosen using jModelTest in PartitionFinder, Gamma
- model of rate heterogeneity and once with the CAT model

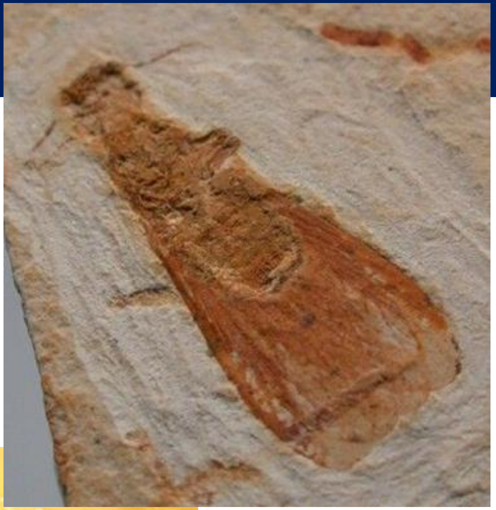
T Davis / InofEscola

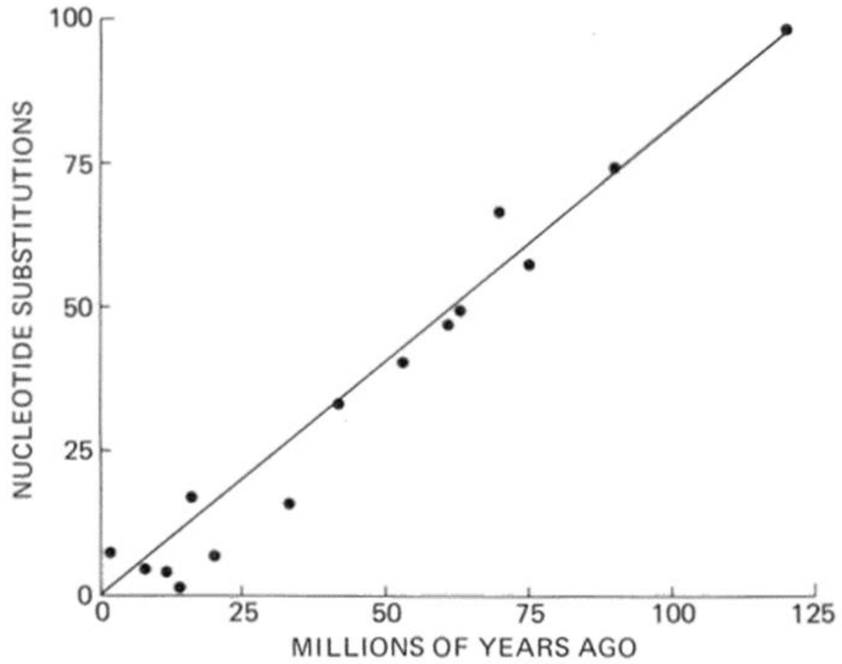
Dating evolutionary events

Dating with a combination of 10 fossil species
Termites: 18 – 137 MYA (radioisotopes, stratigraphy)

Plus 3 other previously dated events
Dictyoptera, Phasmatodea: 140 – 311 MYA

Correlate with base substitutions in mt genomes of modern species = ‘molecular clock’

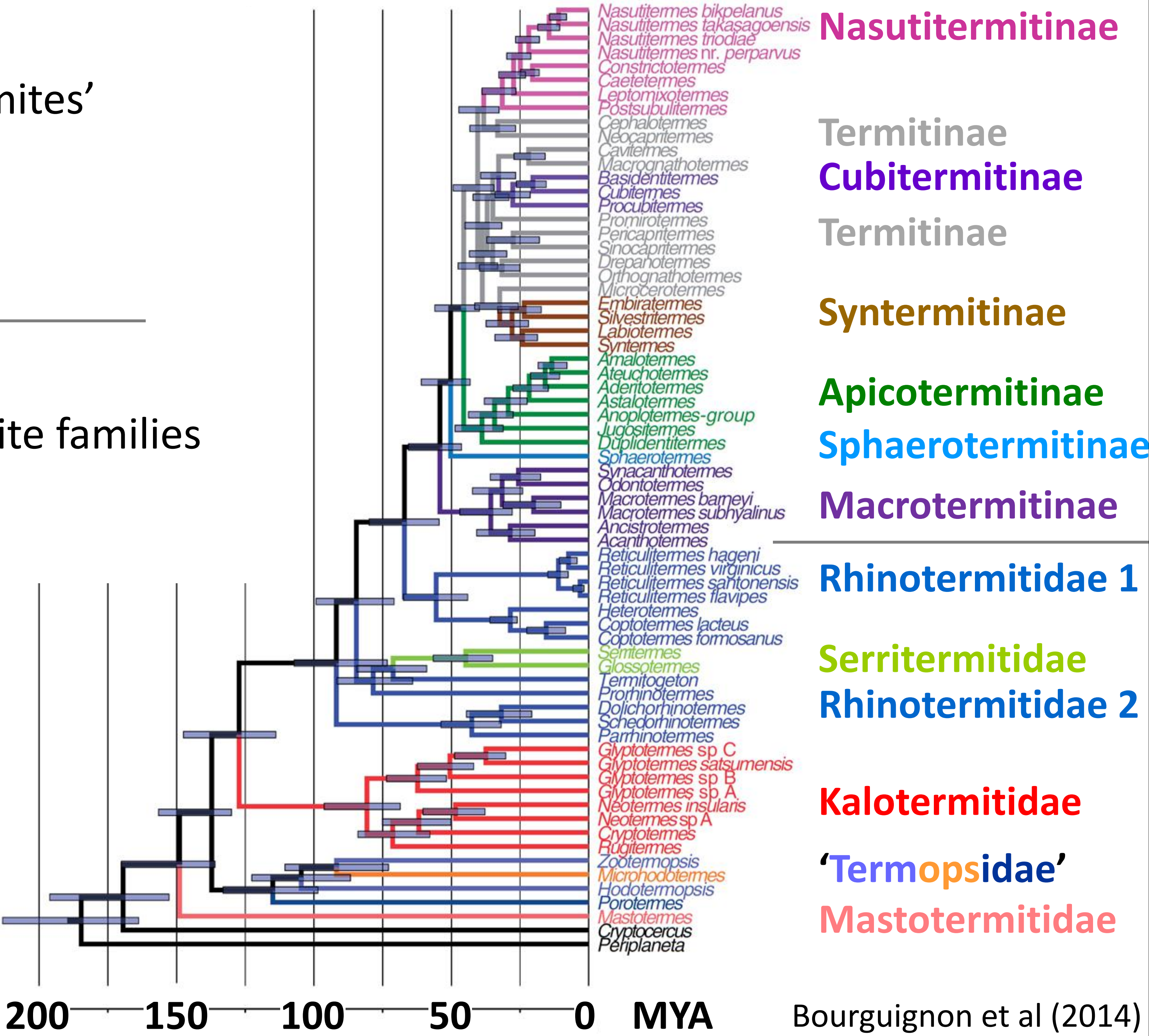




Termitidae
'Higher termites'
subfamilies
'…nae'

Lower termite families
'…dae'

With dates
200 MYA
to present



Bourguignon et al (2014)

Dating termite evolution

Termites evolved from cockroaches ca. 175 MYA

Mastotermes ca. 150 MYA - really is a living fossil

Termopsidae (dampwoods) ca. 140 MYA

Kalotermitdae (drywoods) ca. 125 MYA

Rhinotermitidae is really two families
- *Schedorhinotermes* etc ca. 90 MYA
- *Reticulitermes*, *Heterotermes*, *Coptotermes* ca. 70 MYA

Termitidae (higher termites) ca. 50 MYA
- *Macrotermes* fungus growers first ca. 50 MYA
- Apicotermitid soil feeders second ca. 45 MYA
- Multiple evolutions of snapping, chemical defences
- *Nasutitermes* glue guns last ca. 32 MYA

Bourguignon et al (2014) *Mol Biol Evol* **32**, 406–421

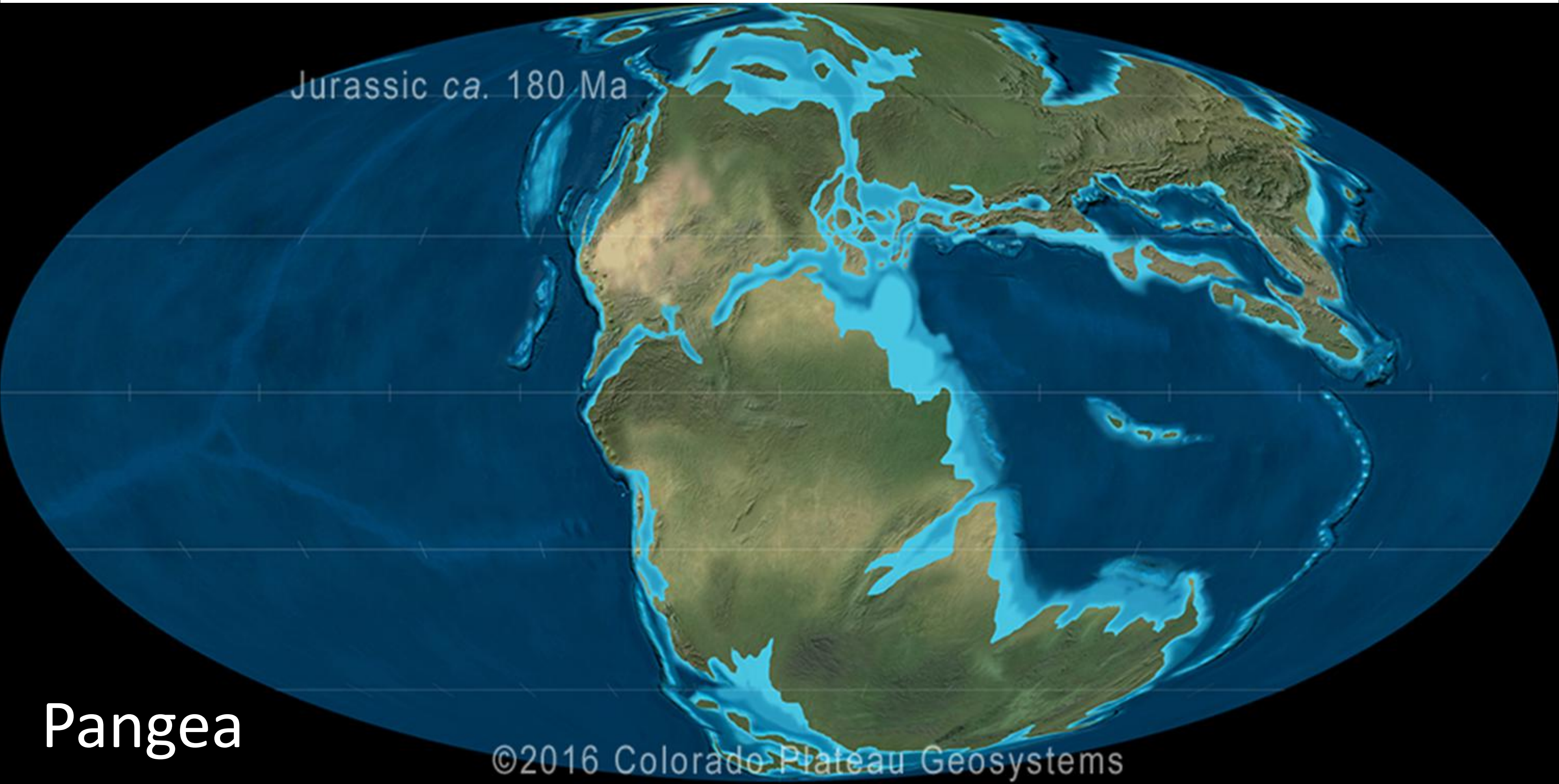
Jurassic ca. 180 MYA

Origin of termites:

Pangea = single land mass / all modern continents joined together

Mastotermes and Termopsidae (dampwood termites) could disperse

Fossils on all continents



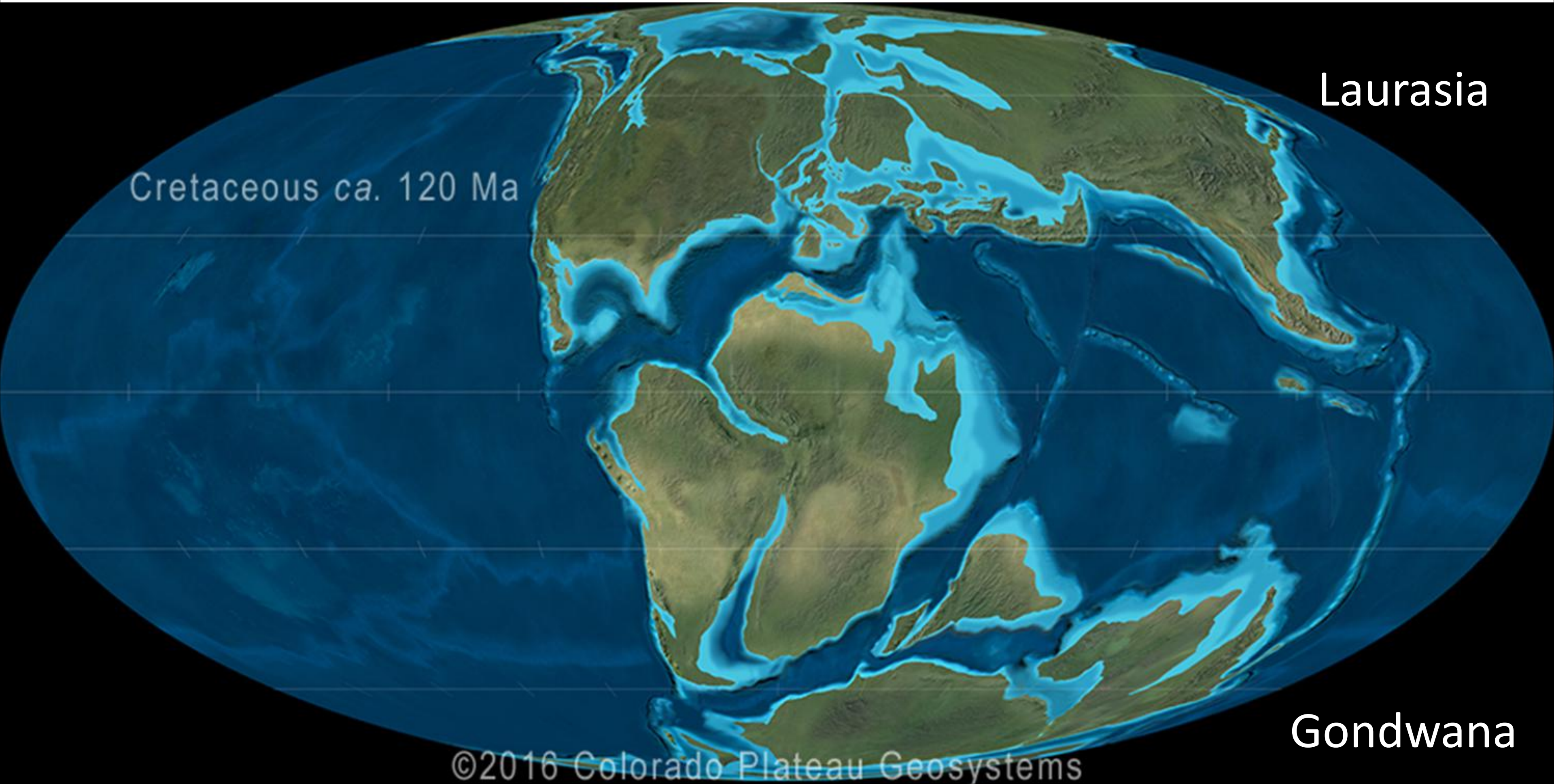
Cretaceous ca. 120 MYA

Origin of Kalotermitidae (drywood termites):

Pangea separated, Gondwana (southern continents) mostly joined still

Dispersal difficult due to oceans, require dead wood as rafts

Fossils on all continents



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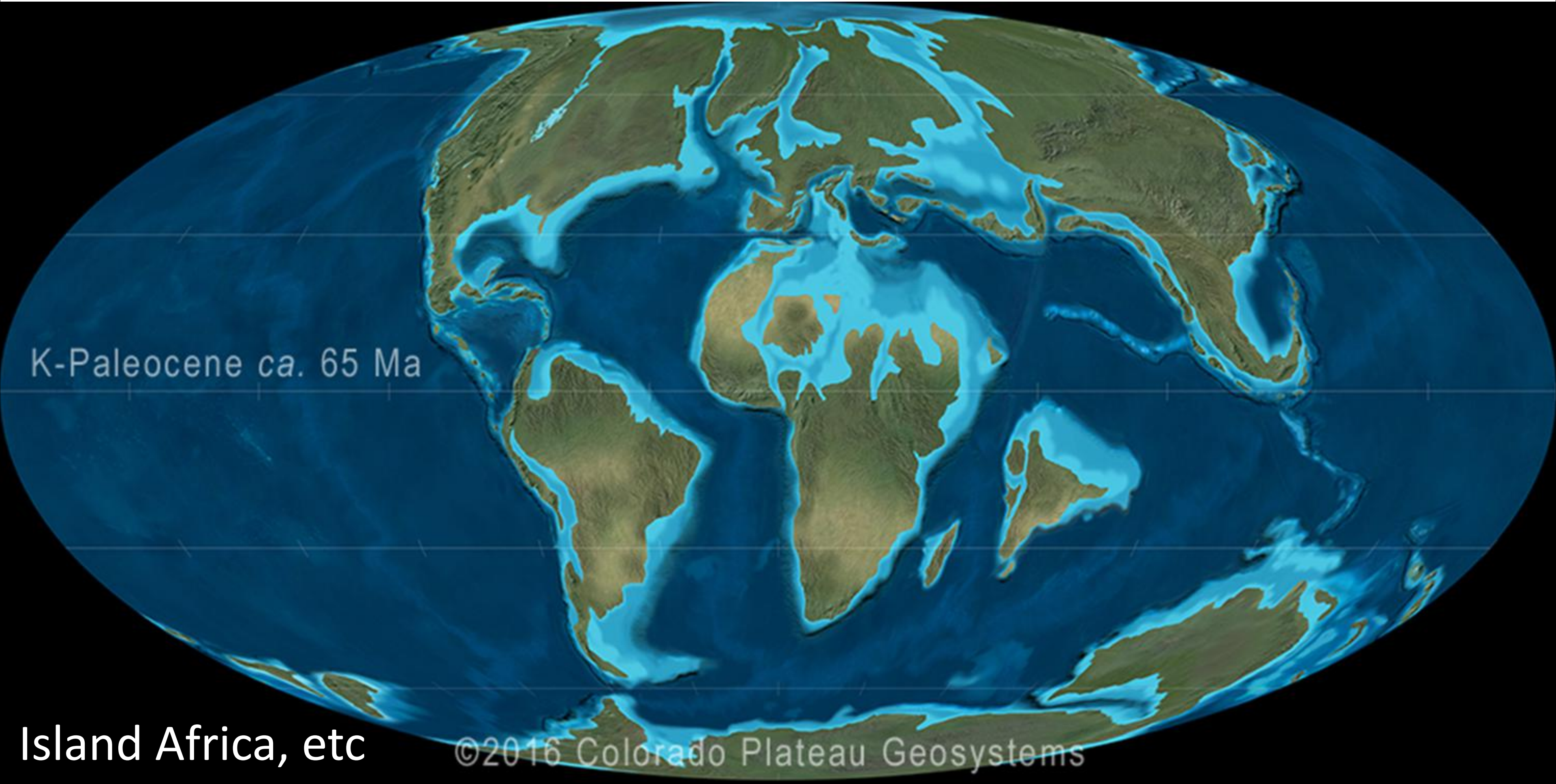
Cretaceous mass extinction ca. 65 MYA

End of dinosaurs / Origin of Rhinotermitidae 2, incl. *Coptotermes*

Gondwana mostly separate: island Africa & island India

Dispersal more difficult (oceans wider)

Fossils on some continents



Island Africa, etc

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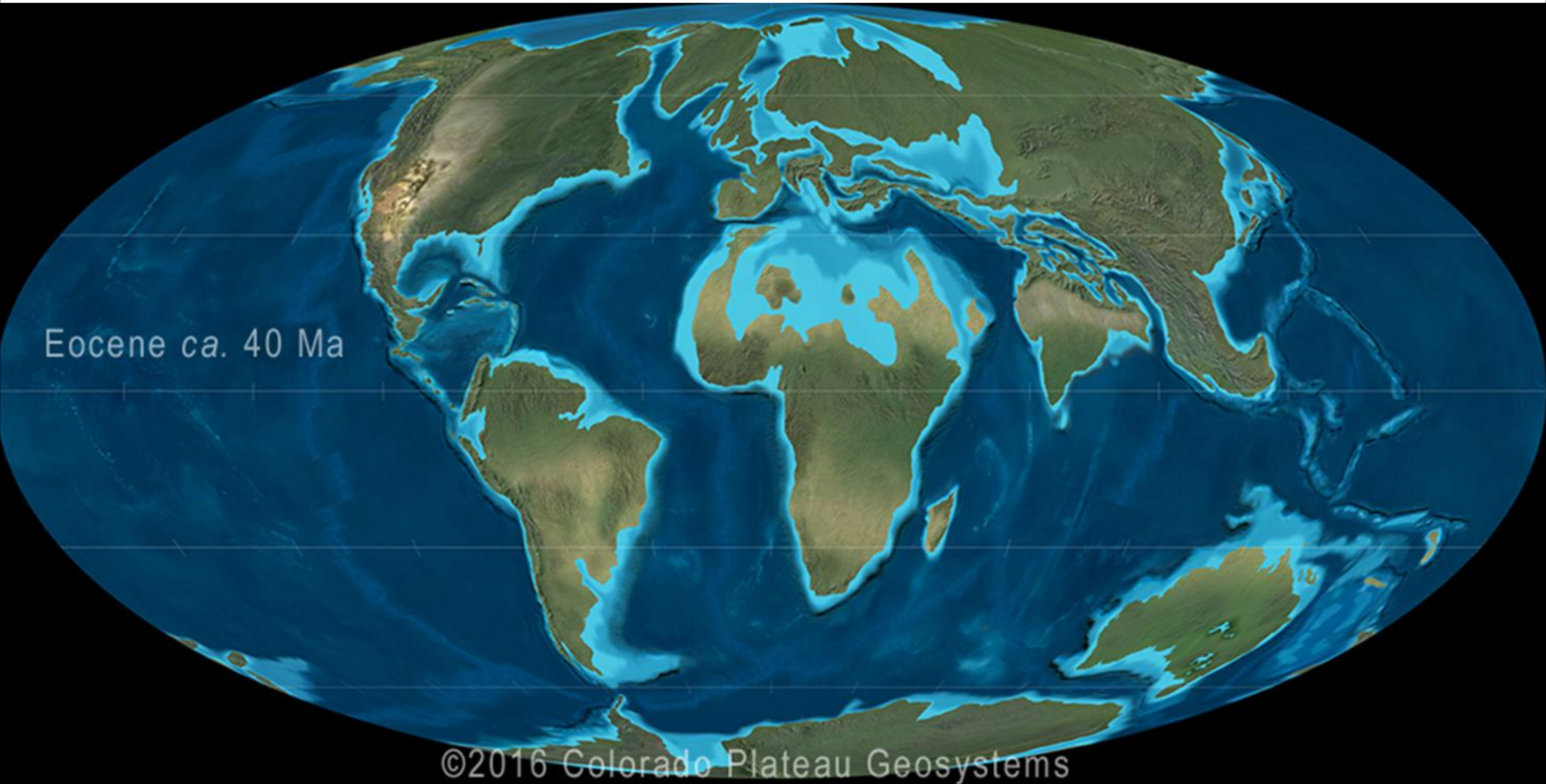
Eocene ca. 40 MYA

10 MYA after origin of Termitidae (higher termites)

All continents separate, though India joining Asia (35 MYA); Americas, Africa+Eurasia nearing

Dispersal getting easier in places (as oceans narrow)



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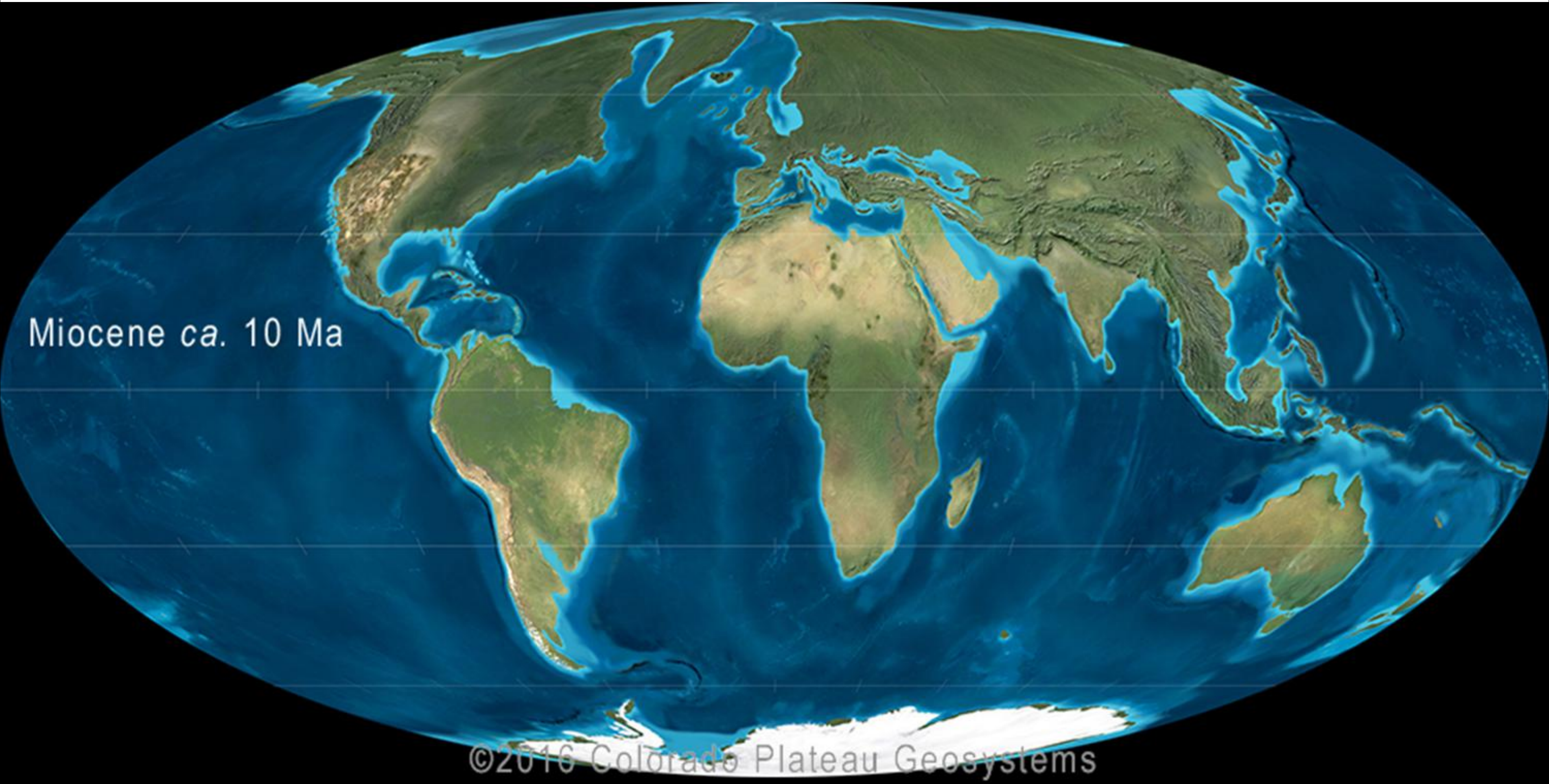
Miocene ca. 10 MYA

10 MYA after origin of Termitidae (higher termites)

All continents joined, Americas (7-3 MYA), Africa+Eurasia (18-20 MYA)

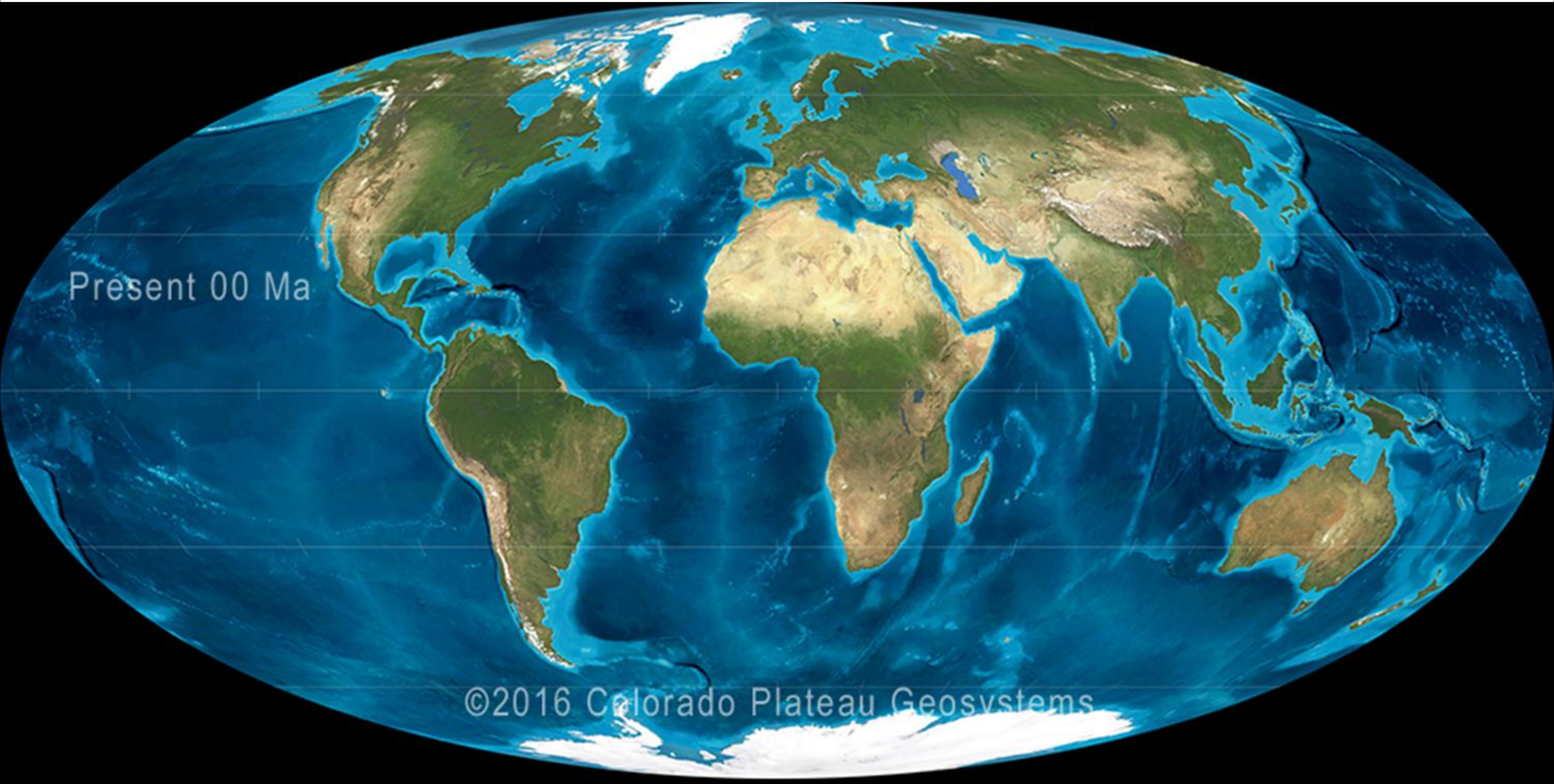
Dispersal getting very easy in places (as oceans now very narrow)



Present

Americas joined, Africa+Eurasia joined

Dispersal super easy (oceans narrowest), especially Asia to Australia



Study 3: 2015-2017

Third step, estimate the timing and geographic pathways of spread of termites around the world

Part A: Rhinotermitidae 1 (Heterotermitidae) – major pests

- Sequenced mt genomes of 44 new *Reticulitermes*, *Heterotermes* & *Coptotermes* species
- Included 14 previous species

Part B: Termitidae – ‘higher termites’ = 70% of all species

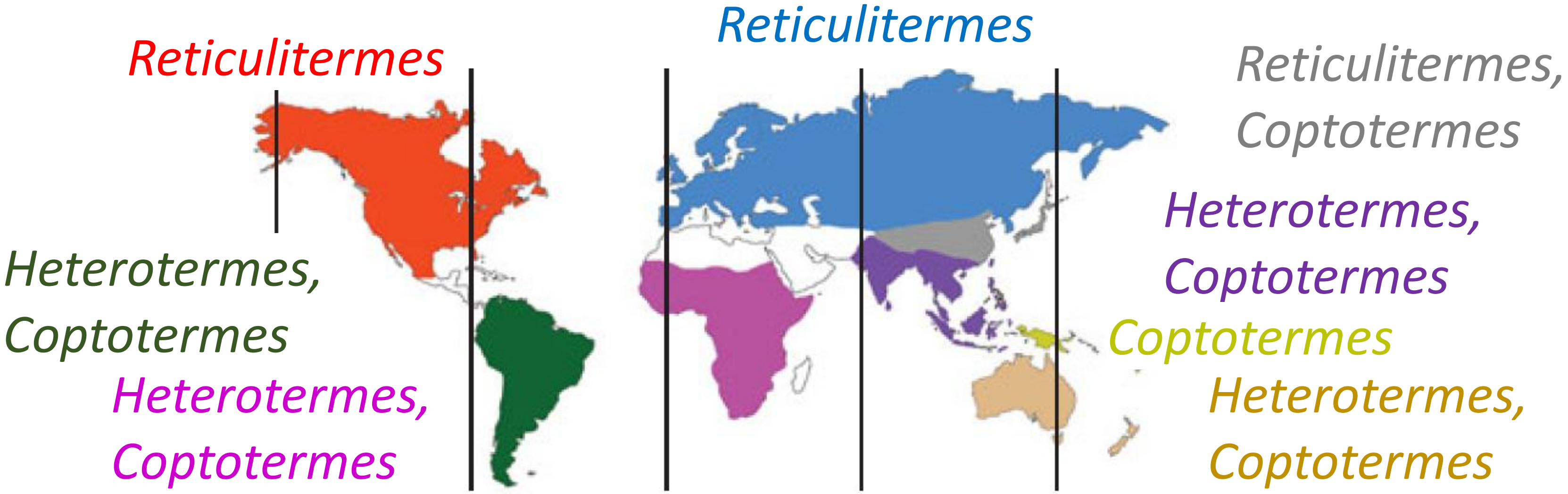
- Sequenced mt genomes of 349 new termite species
- Included 66 previous termite species
- Added 5 mt genomes of 4 cockroach species & 1 mantid species
- Plus 3 mt genomes of outgroups: heelwalker, phasmid & grasshopper

Bourguignon et al (2016a) *Proc R Soc B* **283**, 20160179;
Bourguignon et al (2016b) *Mol Biol Evol* **34**, 589–597

Focus on Rhinotermitidae 1 evolution

Many pest species in the Rhinotermitidae 1
Esp. *Coptotermes*, *Heterotermes*, *Reticulitermes*, *Schedorhinotermes*

- Clade ca. 70 MYA
- At end of breakup of Gondwana
- All continents mostly separated

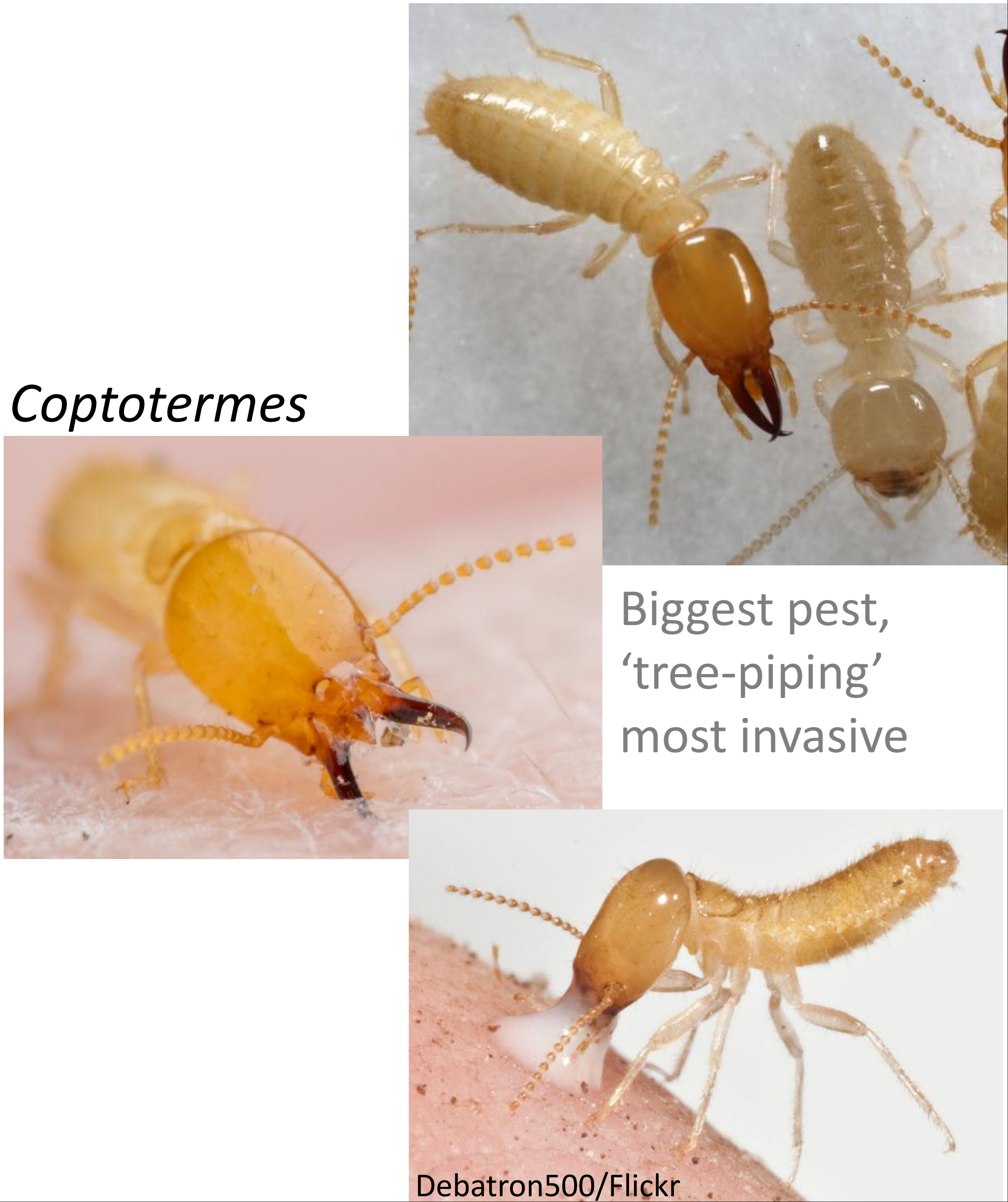


Focus on Rhinotermitidae evolution

Reticulitermes

Heterotermes

Coptotermes



Biggest pest,
'tree-piping'
most invasive

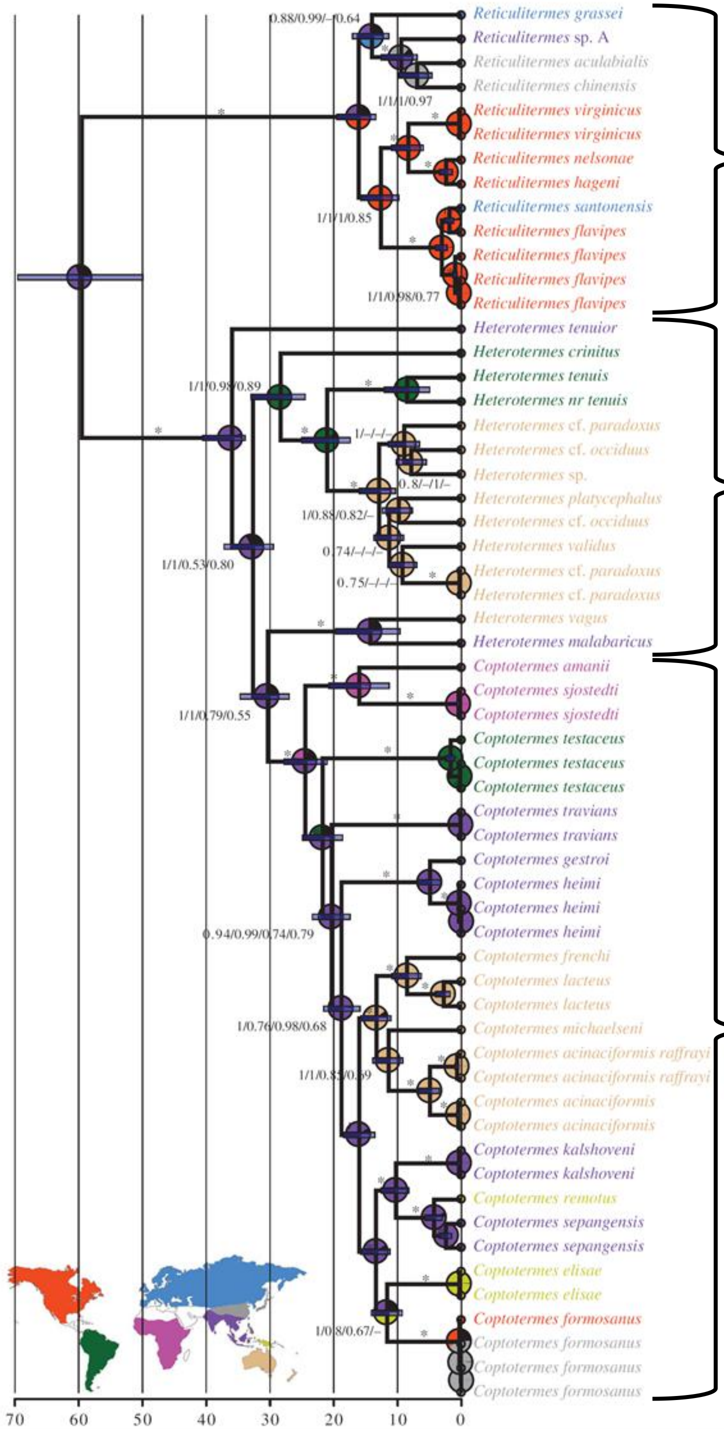
Debatron500/Flickr

Photos by Jan Šobotník, Quah

Rhinotermitidae 1 / Heterotermitidae

With dates
70 MYA
to present

Lineages colour coded to continents



Reticulitermes

Heterotermes

Coptotermes

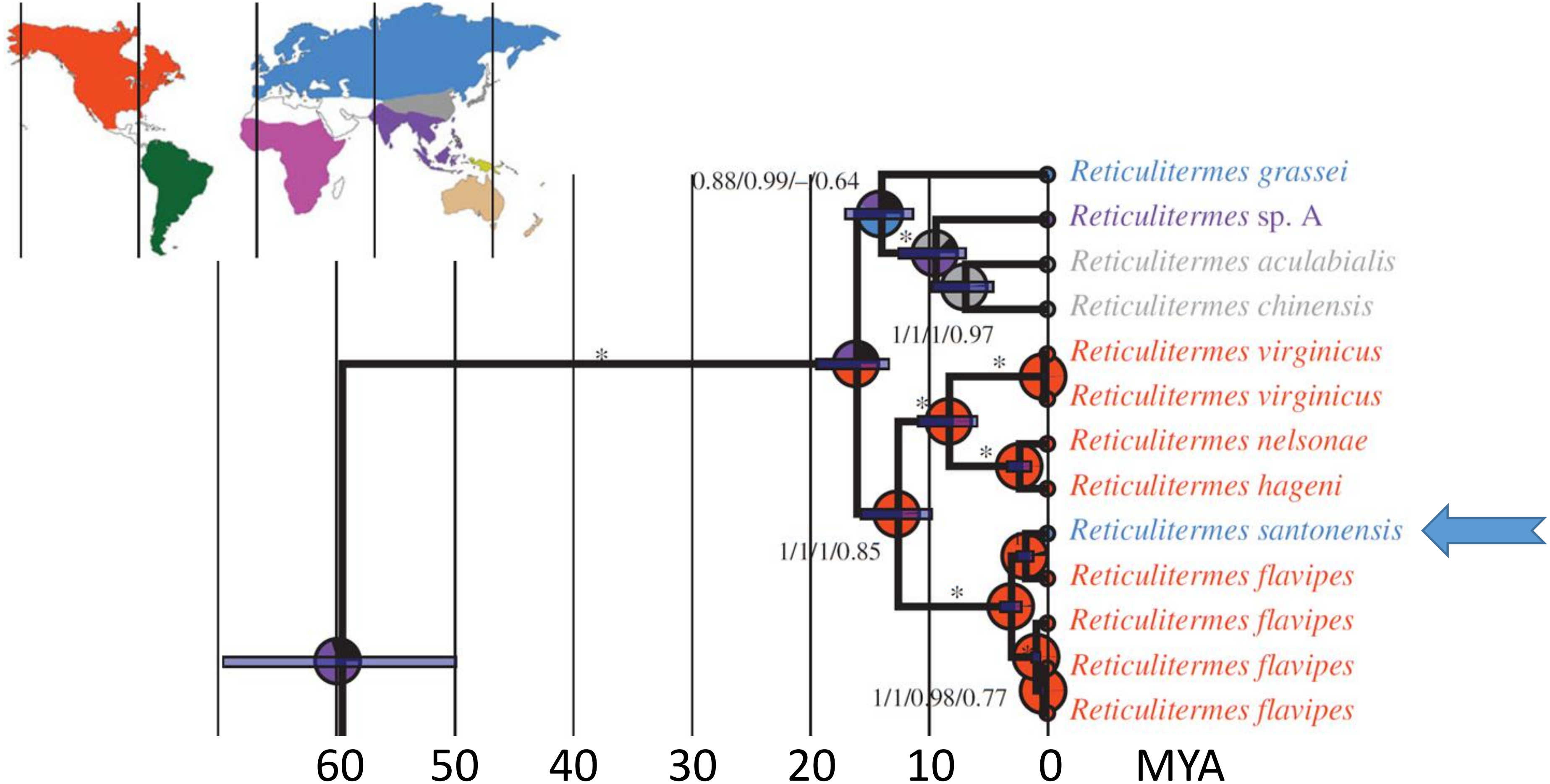
Bourguignon et al (2016a)

Reticulitermes

Most basal lineage in Rhinotermitidae 1 / Heterotermidae

However, *Reticulitermes* is relatively recent, ca. 18 MYA

Only northern hemisphere, Chinese, Indian species most recent



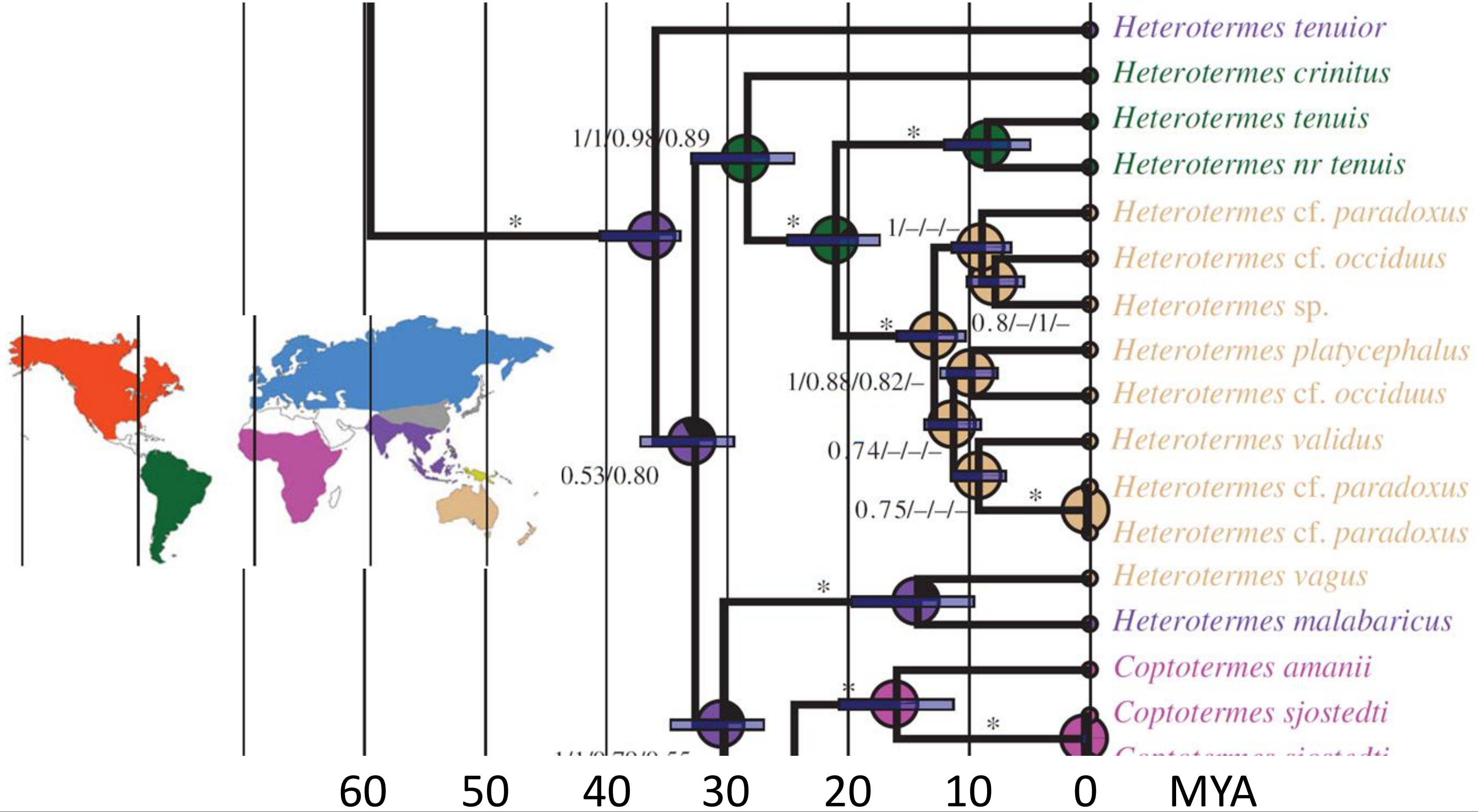
Heterotermes

Middle lineage in Rhinotermitidae 1

However, *Heterotermes* is relatively older, ca. 35 MYA

Indian species oldest (island India?), next S.American,

Australian from S.America & India – two arrivals

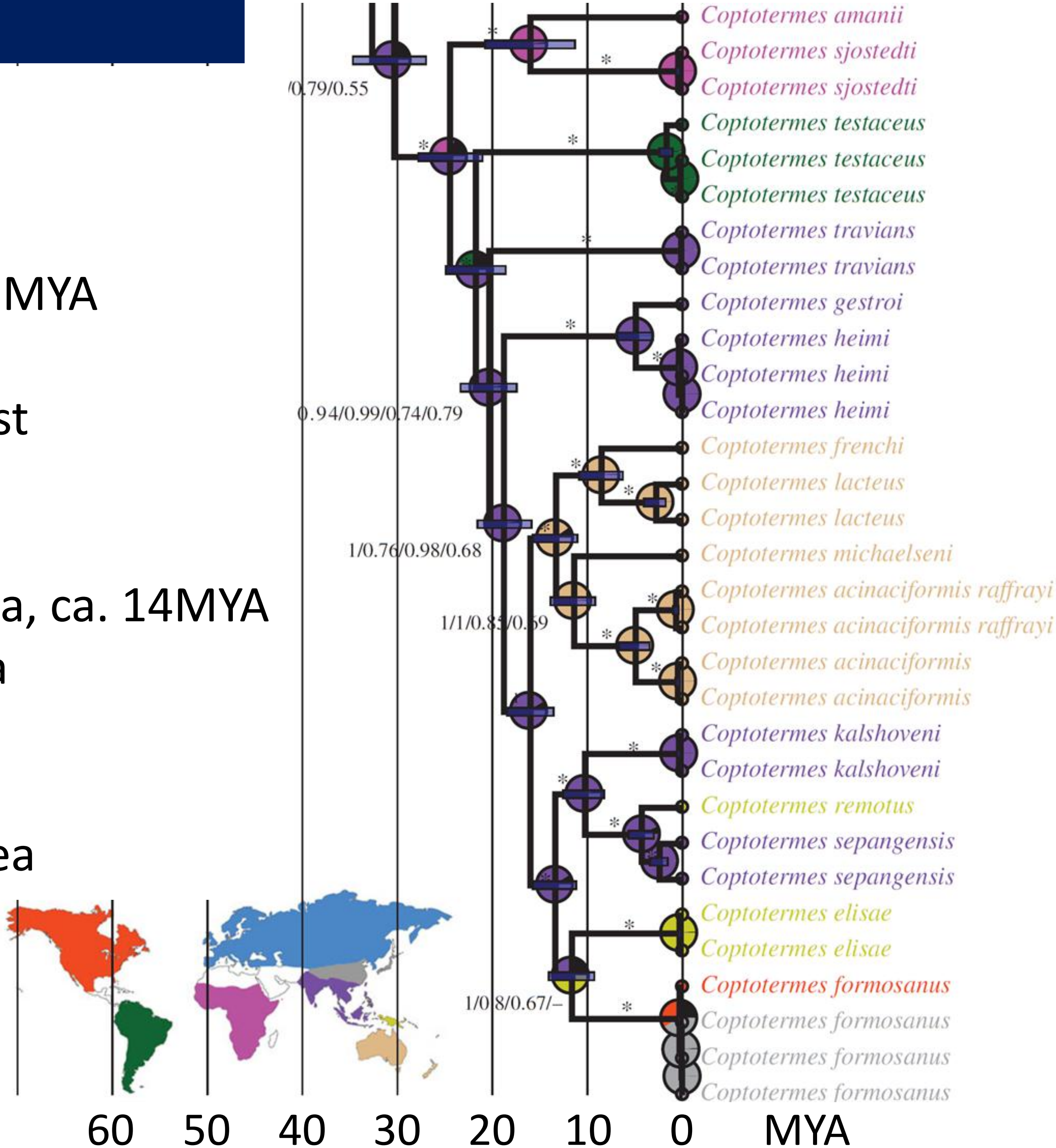


Coptotermes

Latest lineage in Rhinotermitidae 1

Coptotermes is ca. 25 MYA

- Africa species oldest
- Next S.American
- Next Asia
- Australian from Asia, ca. 14MYA
- N.Guinea from Asia
  – 2 arrivals
- China from Asia
  - closest to N.Guinea



Focus on higher termite evolution

More species in the Termitidae = more data

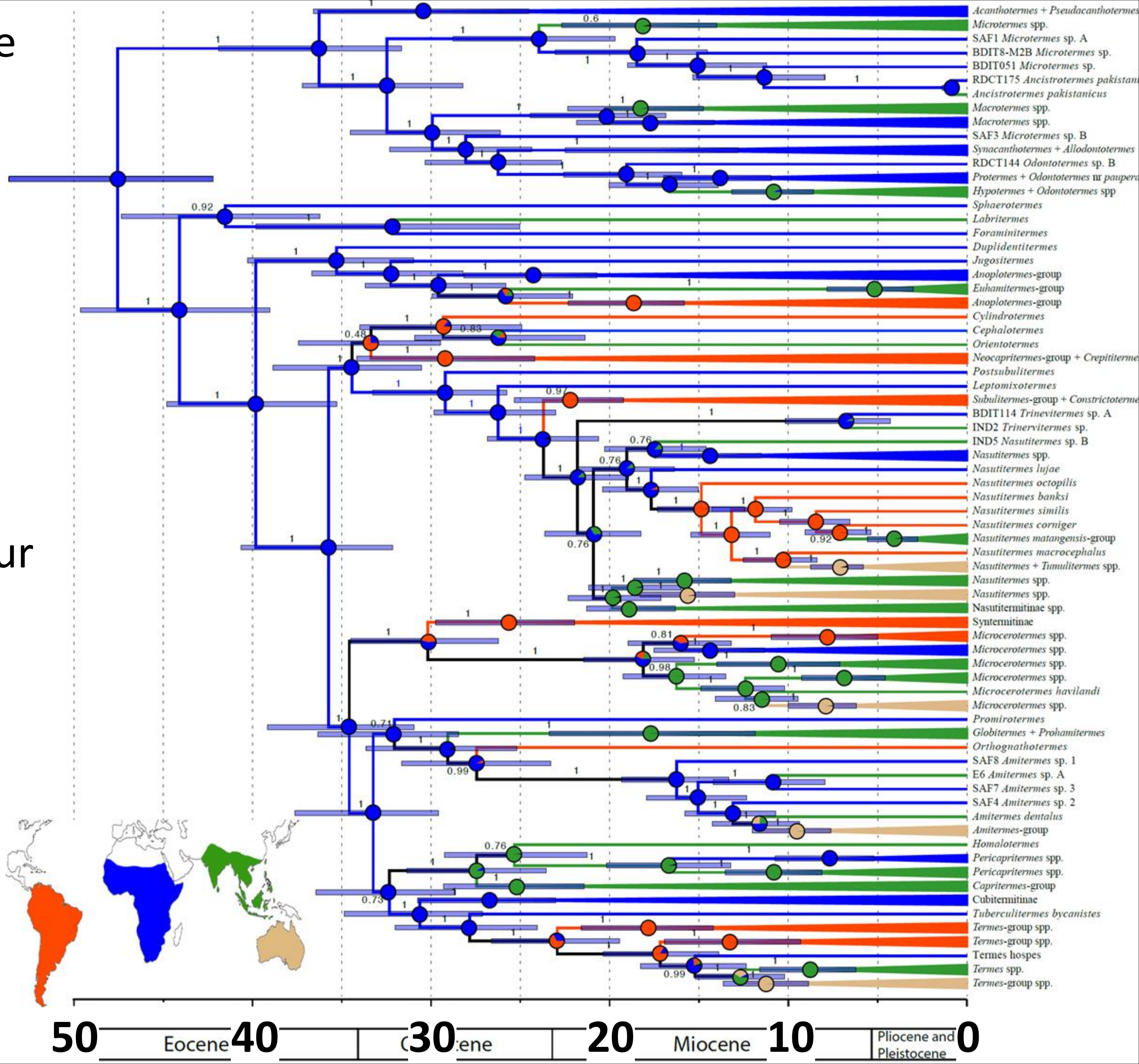
Termitidae evolved in critical period of interest ca. 50 MYA

- *Macrotermes* first ca. 50 MYA
- Termitinae ca. 40 MYA
- *Nasutitermes* last ca. 30 MYA

Higher termite subfamilies & genera

With dates 50 MYA to present

Lineages colour coded to continents



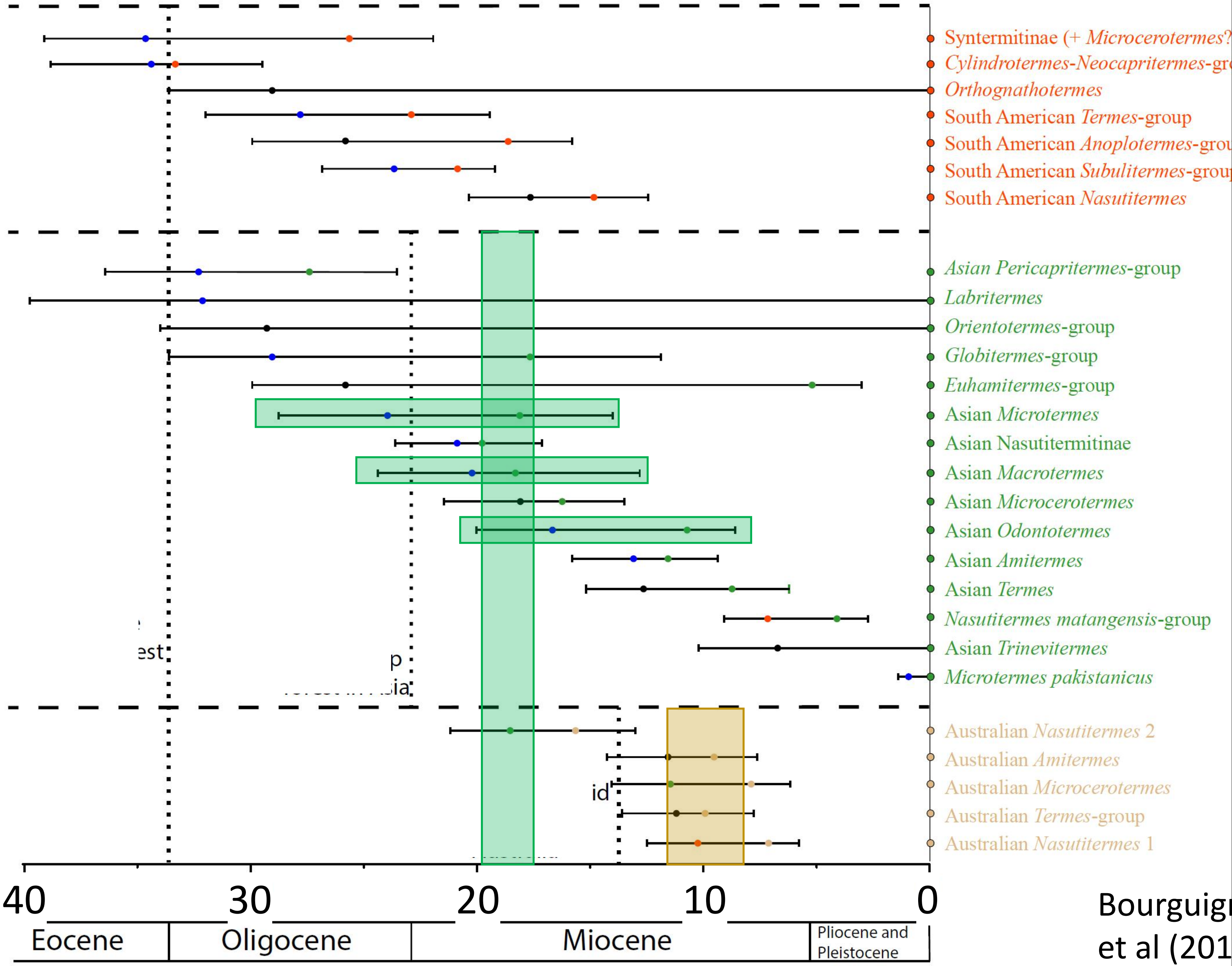
Bourguignon et al (2016b)

Higher termite dispersal

To S.America

To Asia

To Australia



Bourguig
et al (201

Termite evolution and continents

Africa was an island ca. 60 – 20 MYA

Higher termites originated on Africa 50 MYA
- Note the blue throughout the basal part of the phylogram
- Long time to evolve in isolation, allows diversification

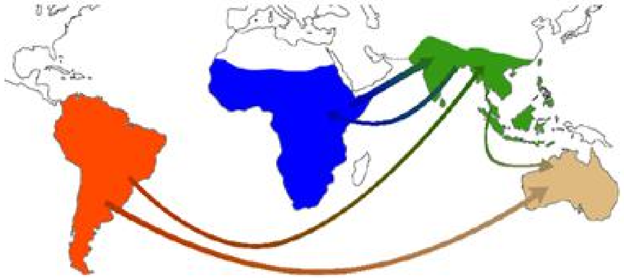
Then they dispersed to other continents

Timing dependent on ocean distance and currents

South America earlier, all oceanic
Eurasia later, some oceanic, some terrestrial
Australia recent, all oceanic

> 35 Ma

≈ 35-15 Ma



≈ 15-0 Ma

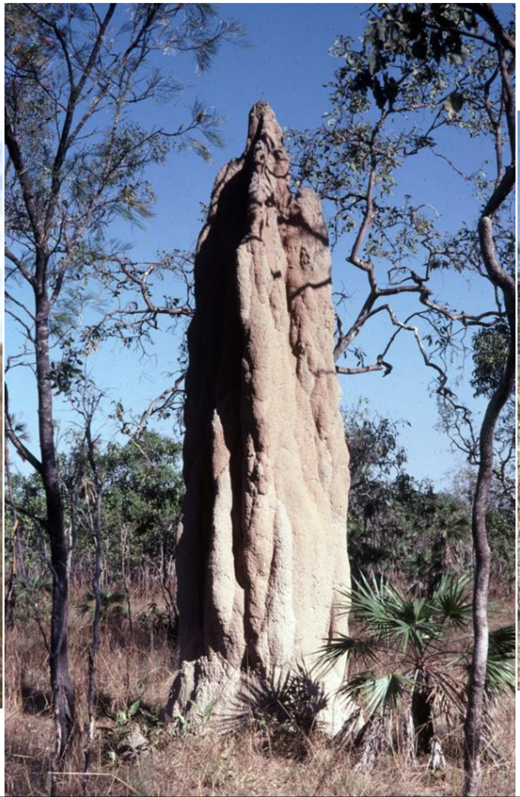
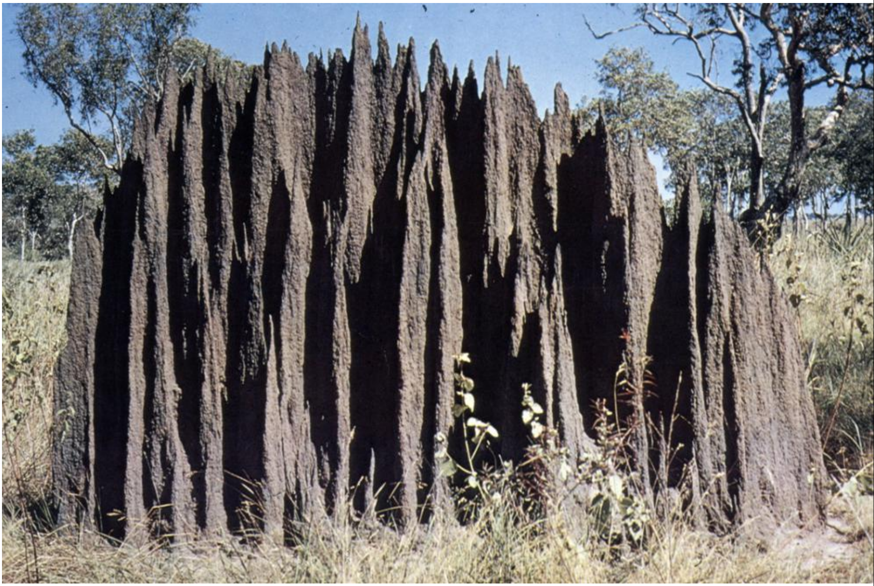
Termite evolution and Australia

Australia had few, basal termites until 15 MYA

- *Mastotermes*
- Termopsidae dampwood termites
- Kalotermitidae drywood termites

Coptotermes very diverse and abundant; arrived ca. 15 MYA

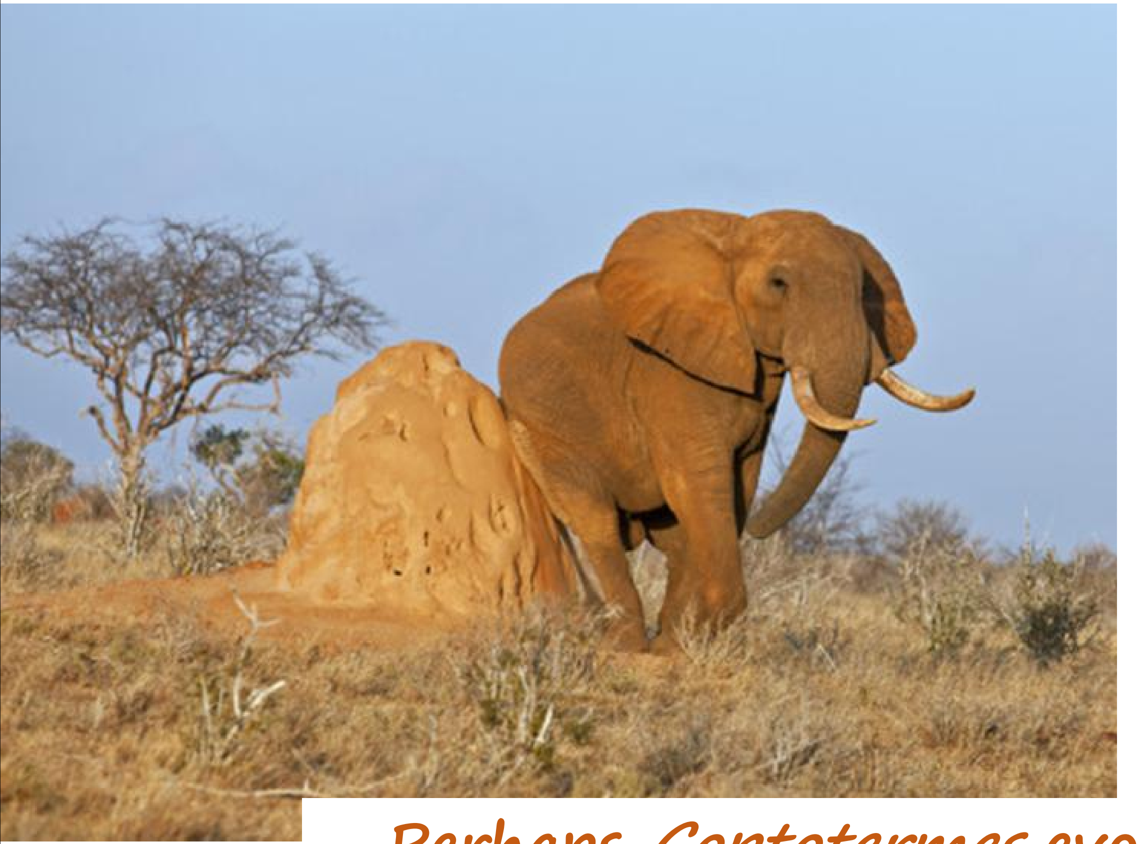
Iconic mounds built by Termitidae 'higher termites' recent < 10 MYA

Coptotermes evolution & competition

Coptotermes evolved in Africa 25 MYA

Fungus growing termites, *Macrotermes*, *Odontotermes*, dominant in Africa, from 50 MYA



Perhaps Coptotermes evolved to live in tree trunks to avoid fungus growing termites?

Fungus growers out-compete *Coptotermes*

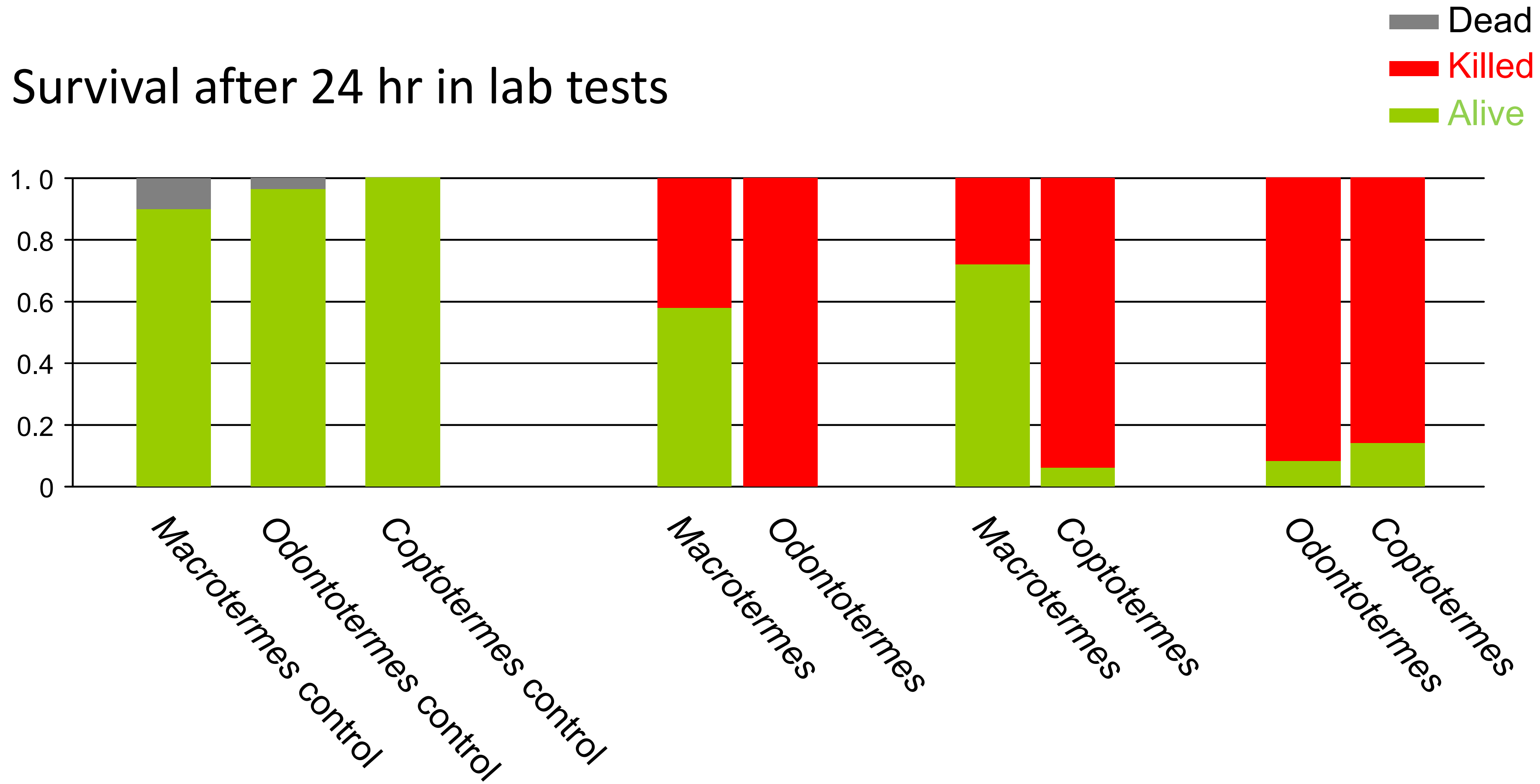
Chinese termites

Relative to *Coptotermes formosanus*:

Macrotermes barneyi – bigger body size, largest colonies

Odontotermes formosanus – similar body size, larger colonies

Survival after 24 hr in lab tests



Evans & Kasseney (2019) *Insects* **10(7)**, 210

Australian *Coptotermes* team

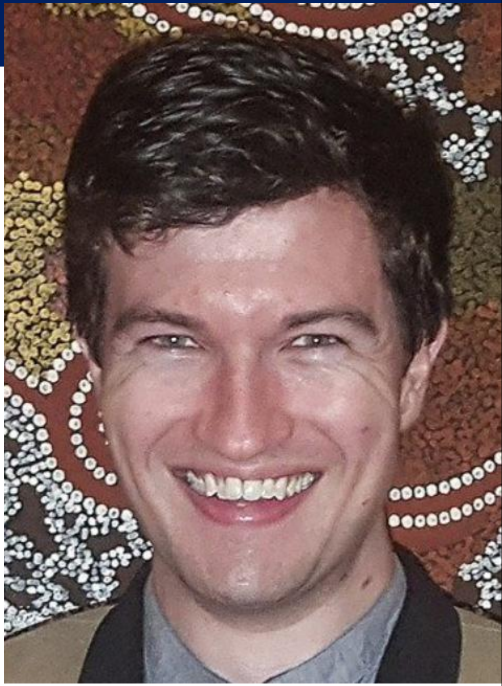
Mt:

- 2100 bp fragment, incl. *cox1*, *trnL* & *cox2*,
- 380 bp portion of *12S*
- 410 bp portion of *16S*

Nuclear:

- 500 bp portion of *ITS1*

Timothy Lee
Univ Sydney/
Aust Museum



Stephen Cameron
CSIRO/
Purdue Univ

Nathan Lo
Simon Ho
Univ
Sydney

Coptotermes & Australian diversity

Fungus growing termites can't raft due to fungus

- Only in Africa & Asia (from 18 MYA)

Elsewhere *Coptotermes* may get release from dominant competitors

- Speciation & diversification

Despite less time, most *Coptotermes* spp in Australia

Continent	Origin mya	# spp
Africa	25	3
Madagascar	?	1
S America	21	1-4?
Asia	20	6
Australia	14	9
New Guinea	11	4

nanus *frenchi* *acinaciformis*

Lee et al (2015) *Mol Phylogen Evol* **82**, 234-244; Lee et al (2017) *Invert Syst* **31**, 180–190

Coptotermes & Australian diversity

Three Australian *Coptotermes* spp. only lower termites in the world to build mounds

Dominant wood-eater in their habitats

Excellent for research purposes

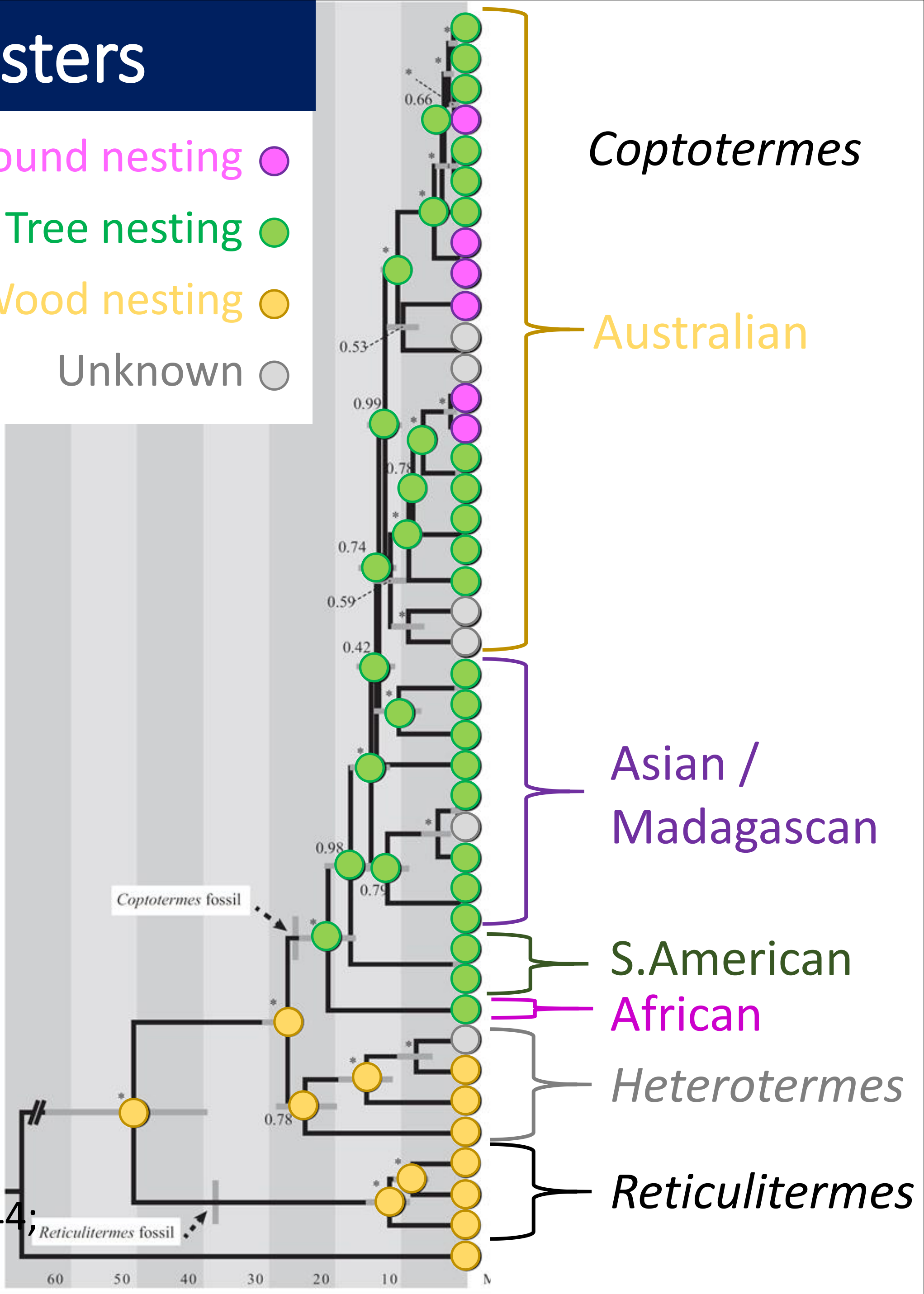
Copototermes are tree nesters

More basal *Reticulitermes* & *Heterotermes* nest in dead wood on the ground

Coptotermes evolved nesting in tree trunks

Eventually, in Australia, *Coptotermes* evolved mound building – 3 times! All very recently

Mound nesting ●
Tree nesting ●
Wood nesting ●
Unknown ○



Lee et al (2015) *Mol Phylogen Evol* **82**, 234-244;
Lee et al (2017) *Invert Syst* **31**, 180–190

Mammals & termites

Alan Root / Getty

Adriano Gambarini

Nicholas Smythe / Getty

Save the Bilgy Fund

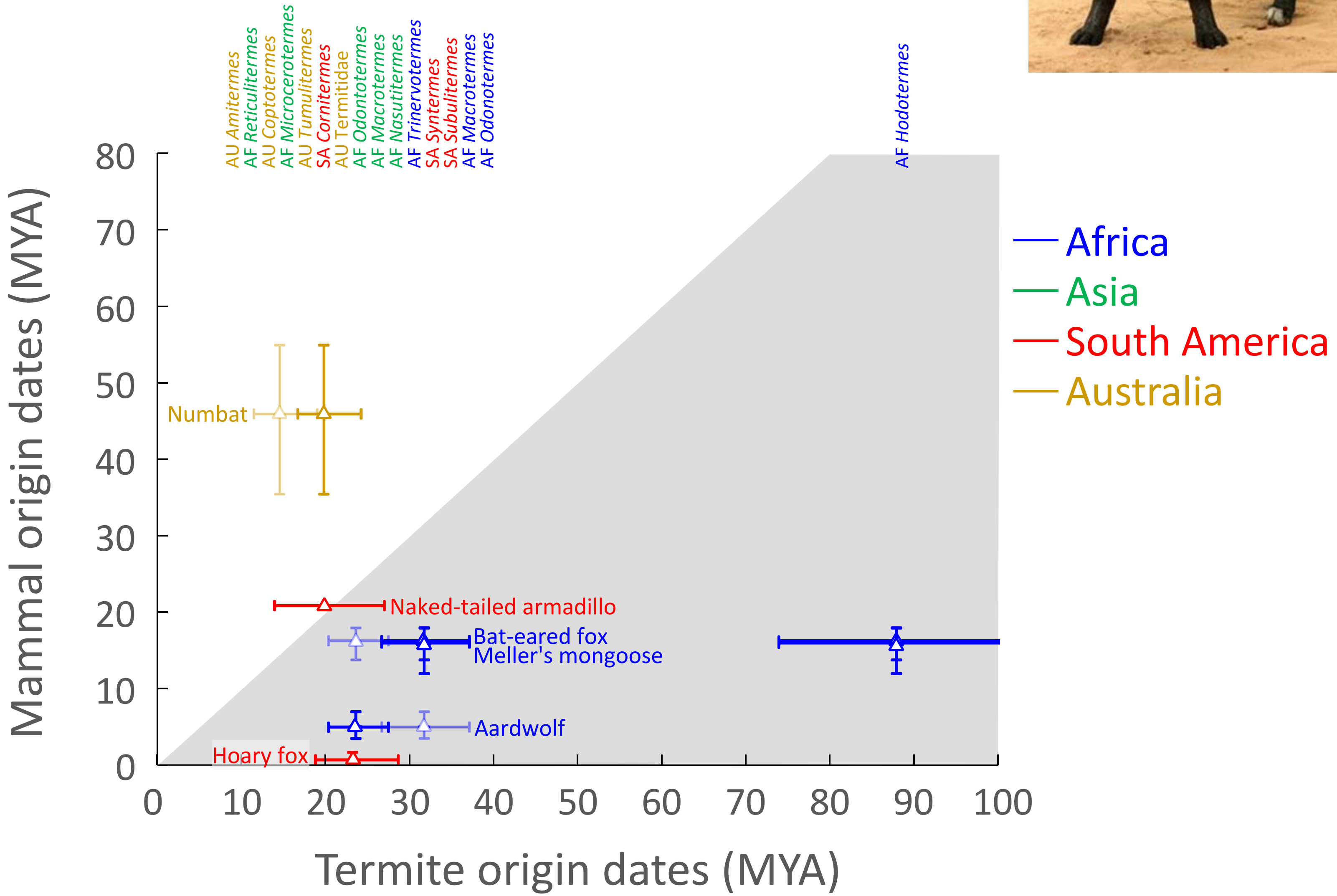
Kalyan Varma

Bunyapon Kusolkrong

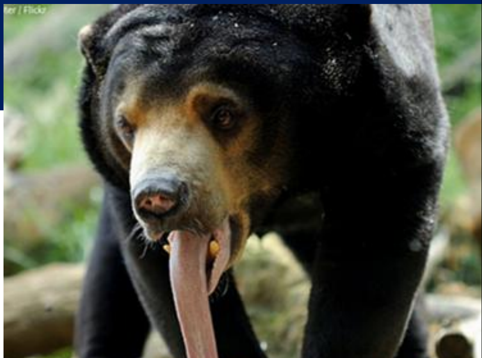
Mammals & termites

Only insects on which mammals have specialised are ants and termites. Which more important?

All termite specialists evolved after their main prey genera

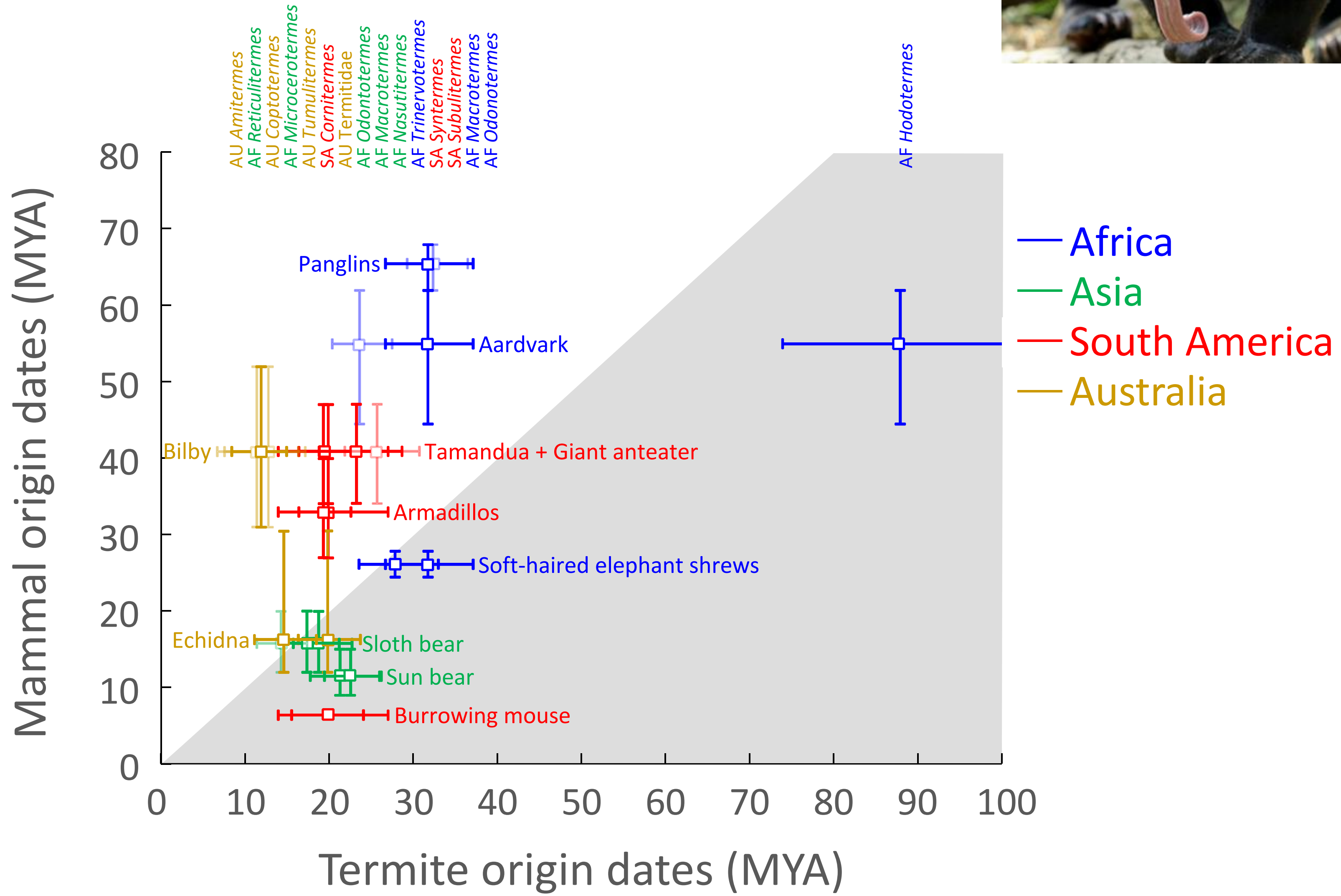


Mammals & termites & ants



Many termite & ant specialists evolved before their main prey termite genera

So likely evolved to eat ants first



Thanks!

Thomas Bourguignon, Tim Lee,
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