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Letter

Reply to Wang & Mao (2015): Molecular dates must be independently testable

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We appreciated Wang & Mao's (2015) constructive commentary on our recent *Tansley Insight* feature (Wilf & Escapa, 2015). There is much in their piece that should be of broad interest to molecular biologists and to paleontologists who wish to learn about new directions in molecular dating. However, true to their title, Wang & Mao confounded our straightforward results and interpretations with a long list of 'puzzling' and 'complicated' statements, disputing nearly every aspect of our paper. Here, we first address broader issues raised regarding the critical need for geologic testing of molecular dates, the importance of standards and collaboration for documenting the timeline of Earth History, and the Green Web biogeographic concept. We then rebut Wang & Mao's specific criticisms of our paper and flatly reject their assertion that our views are "partial and misleading." Like nearly all our paleontological colleagues (and not as Wang & Mao portrayed us), we want molecular dating to work and have eagerly collaborated on productive ideas (e.g., Crisp *et al.*, 2009; Sauquet *et al.*, 2012).

Unfortunately, Wang & Mao's piece reinforced one of the most frustrating external perceptions of molecular dating: there is apparently 'always a reason' why molecular dates cannot be tested independently, no matter what paleontologists and geochronologists do to extract new, high-precision data from undersampled regions of the Earth (Figure 1). There are very few rigorous rock-clock comparisons (see Donoghue & Benton, 2007), especially for plant lineages (our paper), and there is much evidence that small methodological differences cause enormous variance in divergence-age estimates (Sauquet *et al.*, 2012). These issues must be overcome for molecular dating to become a partner to geology in interpreting evolutionary history. *We ask: what does the molecular dating community consider to be an acceptable, feasible test of molecular dating in terms of fundamental principles of geology?*

If molecular dates cannot be tested geologically, and only through other molecular methods in a semi-closed system, then molecular dating does not yet qualify as a technique of historical science, no matter how advanced the computational algorithms, how comprehensive the genetic data used, or how carefully procedural checklists are followed. It is not enough just to feed each new fossil into the pre-existing framework as Wang & Mao suggested. It is critical to know which algorithms have intrinsic predictive value for defined benchmarks in comparison to chance. Otherwise, it is reasonable to suppose that adjusted node-age estimates derived from new calibration fossils might only introduce new mistakes, and that the multifarious high-profile papers reporting large-scale biogeographic and evolutionary conclusions from molecular dates

contain significant errors. These inaccuracies are likely to be compounded when secondary calibrations are used (see, e.g., Sauquet *et al.*, 2012).

35 There is only one true history of Earth and Life. The scientific standards for determining the absolute ages that anchor our understanding of that history are extremely rigorous, including extensive cross-validation among independent isotopic systems (Gradstein *et al.*, 2012). To date a fossil, the ages of associated, radioisotopically analyzed strata must be integrated with the full stratigraphic toolkit, including correlations from lithostratigraphy, biostratigraphy,
40 paleomagnetic stratigraphy, and much more. We emphasize that this work is usually a collaborative effort among the various specialists involved (Figure 1), who work toward the common goal of correctly interpreting Earth history. As in the rock-clock debate, the different data sources often appear initially to be in conflict. The vital process of cross-checking and validation is what leads to new data gathering, methodological improvements, and whatever else
45 is needed to achieve the most precise possible timeline. Several once-controversial methods, such as isotopic stratigraphy, have achieved widespread acceptance in this way.

 We are pleased to see the field of molecular dating making somewhat better use of geologic data when choosing fossils as calibrations. However, there remains a critical need for the community to join, not to continue standing apart from, the collaborative process described
50 above by developing methods for validating molecular dates to geologic standards. At present, we see an unprecedented proliferation of interpretations based on “chronograms” that meet none of the criteria for establishing timelines. Temporal errors can have serious consequences, including for the conservation of highly threatened species and ecosystems. The molecular results indicating that cycad crown genera are young (Neogene; Nagalingum *et al.*, 2011;
55 Condamine *et al.*, 2015) are already affecting biodiversity research on the extremely threatened African cycads (Yessoufou *et al.*, 2014). As headlined in our paper, the Green Web idea (de Queiroz, 2014) is a particularly far-reaching interpretation of molecular dates that we found to be incompatible with the known facts of the fossil record.

 In the short time since our paper was accepted, new data have reinforced our conclusions
60 regarding the Green Web concept. First, a comprehensive study of Gondwanan fossil-rainforest floras from Antarctica, South America, Australia, and New Zealand, and several relevant living assemblages such as the Mt. Kinabalu flora, found that the Gondwanan plant associations are

continuously recognizable from the Late Cretaceous to the present day: before, during, and after Gondwanan breakup (Kooyman *et al.*, 2014). Second, new fossils from Gondwana continue to be much older than previous molecular dates indicated. A spectacular example is the Late Cretaceous pollen grains of basal Asteraceae from Antarctica reported by Barreda *et al.* (2015). Third, molecular dates are used in support of sweeping, potentially inaccurate historical interpretations involving Gondwanan floras (and many other topics). One recent example is a genetic survey and molecular clock analysis across the living Kinabalu biota (Merckx *et al.*, 2015). This work concluded that most of the mountain's extraordinary endemic diversity arose after the main phase of uplift (ca. 6 Ma), with profound implications for biological response capacity and extinction scenarios under future climate change. It seems possible that this interpretation at least partly stems from megabiased clocks. The Kinabalu flora is rich in demonstrably ancient Gondwanic elements, most notably the dominant and diverse conifers in Podocarpaceae and *Agathis* (e.g., Beaman & Beaman, 1993; Kooyman *et al.*, 2014). However, Merckx *et al.* only analyzed one conifer species (*Dacrycarpus kinabaluensis*) and used outdated age constraints, instead of the superbly preserved, 52.2 Ma *Dacrycarpus puertae* fossils that were available from Patagonia (Wilf, 2012). This constraint undoubtedly would have generated much older dates for *D. kinabaluensis*.

Our more specific rebuttals now follow. First, Wang & Mao contested even our introductory statements about how difficult it has been to test molecular dates. Nevertheless, what we originally stated remains correct. Molecular dates that are truly too old “cannot be falsified because the required fossils never existed,” and dates that are clearly too young are frequently explained away using the “minimum age” argument. Here and throughout their piece, Wang & Mao suggested that the solution is to plug any new fossils into the algorithms as priors and recalculate the nodes. However, this procedure avoids the basic need to test molecular dates independently (e.g., geologically). Their suggestion only solves the problem near the position of a particular fossil, not the much more important issue that incorrect molecular dates may exist pervasively across published trees that represent numerous clades with limited or no fossil records. One of the most significant potential values of molecular clocks, of course, is the purported ability to at least approximately date lineages with inadequate fossil records.

We had thought that we presented in our paper an extremely straightforward, empirical methodology, in which *we used the geologic ages of 19 Patagonian fossil taxa to independently*

test the most recently published molecular dates of those taxa that, to avoid circularity, did not
95 already use the same fossils as calibrations. We note the timeliness and value of our study, given
the rarity of having available a relatively large number of relatively new fossil taxa from an
undersampled continent for use in this way. Our presentation contrasted with the usual scenario,
wherein each new fossil is ingested into the molecular framework one paper at a time. However,
Wang & Mao disputed our procedure and repeatedly recommended that the fossils instead be
100 used only as calibration priors for recomputed nodes. Obviously, this should, and in all
likelihood will, be done to improve estimates of the relevant node ages, and anyone who wishes
can do so using the data at hand. However, our goal was to test the Green Web concept by
comparing published node ages with completely independent fossil occurrences from Gondwana
that were dated using geochronology. As such, there was no avoiding comparisons between
105 “outdated molecular dates and up-to-date fossil records,” simply because in most cases the
fossils we used had to have been published after the molecular clock studies. Wang & Mao’s
rejection of our simple test makes little sense and appears to negate the scientific method. If new
discoveries can only be appropriated to generate new molecular dates and not to test what came
before, how can molecular dating itself ever be tested? How can molecular dates become a
110 partner to geology if they cannot be compared to the established methods for determining Earth
history?

Wang & Mao took issue with our use of mean molecular dates for comparison to fossil
ages, rather than reported uncertainties. As noted in our paper, these reported uncertainties were
quite variably reported and hard to assess, and often so large as to appear meaningless. Much
115 more importantly, and despite Wang & Mao’s rejection of our methods for a list of genera, the
clear pattern we found among multiple plant lineages was *directional bias of the means towards
young ages*, leaving little interpretive value for the reported uncertainties.

Wang & Mao suggested without evidence that four of the 19 fossil taxa we used, three
Araucaria species and a cycad (Triassic *Kurtziana*), may be ‘outliers,’ apparently because they
120 thought we interpreted some as belonging to crown rather than stem nodes. However, the status
of Middle Jurassic *Araucaria mirabilis* as a crown Araucariaceae is established from extremely
abundant and detailed anatomical evidence and phylogenetic analyses (Escapa & Catalano,
2013), as we discussed. In addition, Escapa & Catalano’s (2013) resolved two Cretaceous (one
Albian, one Albian-Cenomanian) fossil genera in the stem of the agathioid lineage, which is

125 sister to *Araucaria* and contains the living genera *Agathis* and *Wollemia*. Thus, there is
overwhelming support for at least Middle Jurassic (> 163.5 Ma) origins of crown Araucariaceae
and divergence by the early Late Cretaceous (Cenomanian, > 93.9 Ma) of the *Araucaria* and
agathoid clades, in sharp contrast to the considerably younger molecular dates of these lineages
in the papers we cited. For *Araucaria grandifolia* and *Kurtziana*, Wang & Mao's point is
130 misplaced because we considered these as stem (not crown) representatives of their lineages (*A.*
Section *Araucaria* and *Zamiaceae*, respectively). Our provisional placement of Eocene *A.*
pichileufensis in the crown of *A.* Section *Eutacta* may be disputed until a phylogenetic analysis is
done. However, this species is known from abundant, well-preserved cone scales and leafy
branches that are virtually indistinguishable from several extant New Caledonian species in the
135 section.

Wang & Mao even contested our comparatively mundane suggestion, made by others
before us (Bell & Donoghue, 2005), that the standard practice of forcing fossils to stem positions
will cause young bias if their true evolutionary position was near or in the crown. They pointed
out that the reverse is true as well, namely that forcing lineages to a crown will lead to
140 overestimates. However, we did not advocate doing this "radical" reverse technique but only
pointed out the problem caused by the standard practice, calling for a methodological innovation
to address the issue. Indeed, the community seems well on its way towards improving this
situation as Wang & Mao described.

In conclusion, we look forward very much to the day when we will agree with Wang &
145 Mao's assertions that molecular dating "enables the testing of hypotheses about the distribution
and evolution of plants across time and space;" provides "a reliable complement to timescales
based solely on fossils;" and "will be compatible with fossil records by integrating up-to-date
fossil calibrations." Yet, Wang & Mao rejected nearly all our attempts to test these ideas
empirically, despite our presentation of an unprecedented amount of high-quality, diverse
150 geologic and paleontological data. What, then, would Wang & Mao accept as an independent test
of molecular dates? We call on the molecular dating community to recognize the need and to
develop the tests that will better validate the method, using the abundant, outstanding constraint
data that are readily available. Meanwhile, our conclusions stand. Diverse Gondwanan plant
fossils directly refute the Green Web idea for their respective lineages, while clearly showing
155 directional young-bias on corresponding molecular dates. The broader Green Web hypothesis

may well be based on a considerably larger number of directionally biased molecular age estimates for lineages that lack adequate fossil records for comparison.

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Author Contributions

165 Both authors contributed substantially to interpretation of the relevant issues and to writing the manuscript. No new research design or performance, nor data analysis and interpretation, were necessary to compose this Reply.

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220 clocks, paleobotany, Patagonia.



Fig. 1 Geochronology example: the PL-1 tuff (bright white layer at arrow, 2.2 m thick) exposed in the Peñas Coloradas Formation at Palacio de los Loros, Chubut, Patagonia, Argentina (Clyde *et al.*, 2014). Zircons separated from this and two other tuffs discovered in this fossiliferous area each produced similar, high-precision U-Pb ages using laser ablation HR-MC-ICP-MS (high resolution multi collector-inductively coupled plasma mass spectrometry) at the University of Arizona Laserchron Center. Eleven zircons selected from this tuff were then analyzed using high resolution TIMS (thermal ionization mass spectrometry) at the Boise State University Isotope Geology Laboratory, producing a U-Pb age of 61.984 ± 0.041 Ma. Clyde *et al.* (2014) were able to constrain the ages of the diverse and significant plant and animal fossils in several rock formations in this area (including some mentioned in Wilf & Escapa, 2015) for the first time using these results, combined with multiple additional constraints: a new ^{40}Ar - ^{39}Ar age from a nearby Late Cretaceous basalt; new paleomagnetic data; new biostratigraphic data from dinoflagellate cysts, foraminifera, calcareous nannoplankton, and terrestrial palynomorphs; and multiple measured stratigraphic sections. Analyzing each of these sources of data required a separate effort by a dedicated specialist or specialist team.