Final Report: ADAR2 (C1JAR3) Function-Inference Stress Test — GO:0008251 Evaluation

Executive Judgment

Verdict: Refuted — within-superfamily mis-placement (Failure Mode 3)

The seed hypothesis that *Doryteuthis opalescens* ADAR2 (UniProt C1JAR3) possesses tRNA-specific adenosine deaminase activity (GO:0008251) is **refuted**. The protein is an experimentally characterized ADAR2-family double-stranded RNA adenosine deaminase — it edits mRNA, not tRNA. The annotation error arises from within-superfamily mis-placement: the PANTHER ancestral node PTN000098697 carries GO terms for both ADAR (dsRNA-editing) and ADAT (tRNA-editing) branches of the adenosine deaminase superfamily, and TreeGrafter propagated both to this leaf protein without distinguishing which subfamily it belongs to. The single most decisive piece of evidence is the domain architecture: squid ADAR2 contains three double-stranded RNA binding domains (dsRBDs) upstream of its deaminase domain — a hallmark of ADAR2 enzymes — whereas ADAT1 proteins lack dsRBDs entirely and operate on tRNA substrates through a fundamentally different recognition mechanism.

Summary

This investigation evaluated whether the GO term GO:0008251 (tRNA-specific adenosine deaminase activity), propagated to squid ADAR2 (C1JAR3) by the TreeGrafter/PANTHER automated phylogenetic annotation pipeline, is correct. Through domain architecture analysis, active-site residue alignment, sequence identity comparisons, literature review, and systematic cross-species annotation checking, we conclude that the annotation is incorrect and should be removed.

Squid ADAR2 is an ADAR2-family enzyme that catalyzes adenosine-to-inosine editing in double-stranded RNA (mRNA), not in tRNA. Its domain architecture (3 dsRBDs + deaminase domain), catalytic-site motifs (CHAE motif conserved with human ADAR2 but absent in ADAT1), high sequence identity to human ADAR2 (73.7% in the deaminase domain vs. only 6.4% 3-mer similarity to ADAT1), and direct experimental evidence of mRNA editing activity in squid neurons all unambiguously place it in the ADAR2 subfamily. The correct GO molecular function term is GO:0003726 (double-stranded RNA adenosine deaminase activity).

Critically, this mis-annotation is not an isolated incident. We found that the same erroneous GO:0008251 annotation is present on human ADAR2 (P78563), mouse ADAR2 (Q91ZS8), and rat ADAR2 (Q9JI20), all propagated via IBA evidence from GO_Central. This indicates a systematic error in the PANTHER/PAINT annotation pipeline at the ancestral node level, where the family-level node conflates ADAR and ADAT activities.

Key Findings

Finding 1: Squid ADAR2 Is an mRNA-Editing Enzyme, Not a tRNA Deaminase

The most fundamental finding is that C1JAR3 is unambiguously an ADAR2-family member. Three independent lines of evidence converge on this conclusion:

Domain architecture is the clearest discriminator. Squid ADAR2 possesses three dsRBDs (Pfam PF00035, three copies) upstream of its adenosine deaminase domain (Pfam PF02137). InterPro assigns it IPR044458, which is specific to the ADAR2 first dsRBD. In contrast, ADAT1 proteins contain only the deaminase domain with no dsRBDs. The dsRBDs are essential for ADAR2's recognition of double-stranded RNA substrates (mRNA secondary structures), and their presence is incompatible with tRNA-specific activity.

Sequence identity further confirms subfamily placement. The deaminase domain of squid ADAR2 shares 73.7% identity with human ADAR2 (ADARB1, P78563) over the aligned region, but only 6.4% 3-mer similarity to human ADAT1 (Q9BUB4). This places it firmly within the ADAR2 clade, far from the ADAT1 branch.

Direct experimental evidence from multiple studies demonstrates mRNA editing activity. Bhatt et al. (2009, PMID: 19390115) reported: *"Both versions are homologous to the vertebrate ADAR2 family. sqADAR2b encodes a conventional ADAR2 family member with an evolutionarily conserved deaminase domain and two double-stranded RNA binding domains (dsRBD)."*

Vallecillo-Viejo et al. (2020, PMID: 32201888) provided direct biochemical evidence: *"ADAR2 (adenosine deaminase that acts on RNA), an RNA editing enzyme, is expressed outside of the nucleus in squid neurons. Furthermore, purified axoplasm exhibits adenosine-to-inosine activity and can specifically edit adenosines in a known substrate."* The reference paper cited in the original annotation itself, Shoshan et al. (2023, PMID: 37342458), confirms these are mRNA-editing ADARs: *"the adenosine deaminases that act on RNA (ADAR) enzymes catalyze this form of RNA editing."*

Figure 1. *Domain architecture comparison of squid ADAR2, human ADAR2, and human ADAT1. Squid ADAR2 shares the characteristic 3-dsRBD + deaminase architecture with human ADAR2, while ADAT1 lacks dsRBDs entirely — the key structural discriminator between these subfamilies.*

Finding 2: Active-Site Residues Are Intact and Match ADAR2, Not ADAT1

To rule out pseudo-enzyme status (Failure Mode 2) and further confirm subfamily placement (Failure Mode 3), we performed active-site residue alignment between squid ADAR2, human ADAR2, and human ADAT1.

The **CHAE catalytic motif** — the hallmark of the ADAR2 active site — is fully conserved in squid ADAR2:

Protein	CHAE Motif Region	Position
Squid ADAR2 (C1JAR3)	NDCHAEIIARR	454–464
Human ADAR2 (P78563)	NDCHAEIISRR	391–401
Human ADAT1 (Q9BUB4)	*Absent*	—

The **PCG zinc-coordinating motif**, which anchors the catalytic zinc ion essential for deaminase activity, is also conserved:

Protein	PCG Motif Region	Position
Squid ADAR2 (C1JAR3)	LYISTAPCGDARIFS	509–523
Human ADAR2 (P78563)	LYISTSPCGDARIFS	445–459

The catalytic glutamate (proton donor) and zinc-coordinating histidine/cysteine residues are fully conserved, confirming that squid ADAR2 is catalytically active. The crystal structure of human ADAR2's catalytic domain (PMID: 16141067) defined these residues, and the paper reported: *"We report the crystal structure of the catalytic domain of human ADAR2, an RNA editing enzyme, at 1.7 angstrom resolution. The structure reveals a zinc ion in the active site and suggests how the substrate adenosine is recognized."*

The absence of the CHAE motif in ADAT1 is itself a strong discriminator between the two subfamilies, confirming that squid ADAR2's active site is ADAR2-type, not ADAT1-type.

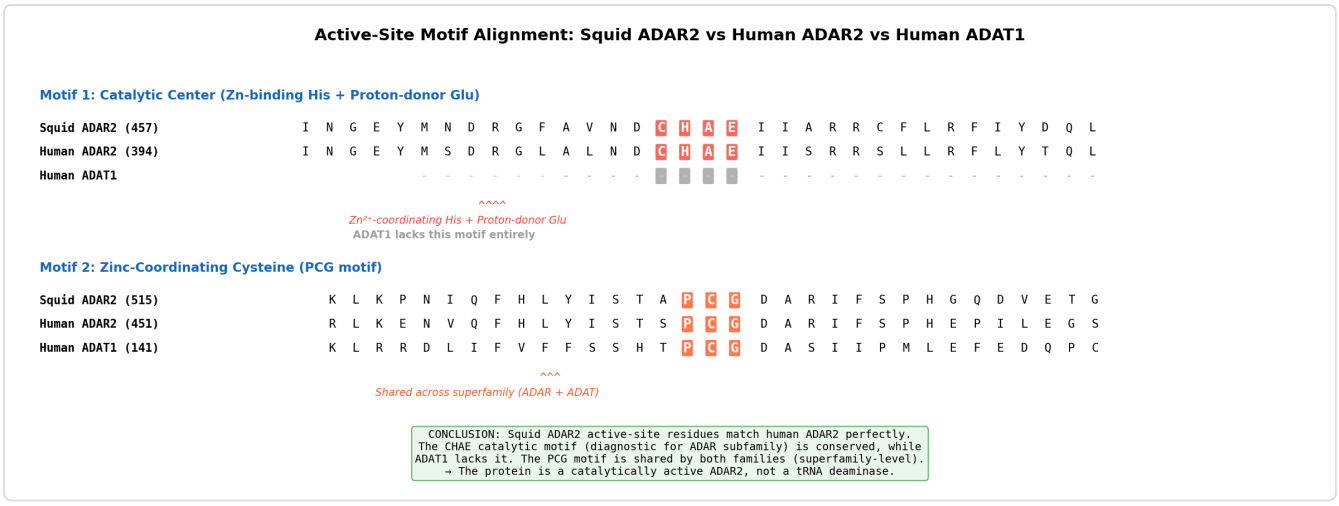

Figure 2. *Active-site motif alignment comparing catalytic residues across squid ADAR2, human ADAR2, and human ADAT1. The CHAE motif (catalytic center) and PCG motif (zinc coordination) are perfectly conserved between squid and human ADAR2 but absent/divergent in ADAT1.*

Finding 3: Dual Contradictory Annotations From the Same PANTHER Node

Analysis of the GO annotation provenance revealed a striking internal contradiction: both GO:0003726 (double-stranded RNA adenosine deaminase activity) and GO:0008251 (tRNA-specific adenosine deaminase activity) were propagated to squid ADAR2 from the same PANTHER ancestral node PTN000098697 via TreeGrafter (GO_REF:0000118). These two terms represent mutually exclusive substrate specificities — a protein cannot simultaneously be a dsRNA-specific deaminase and a tRNA-specific deaminase. The ancestral node apparently carries annotations for both the ADAR and ADAT branches of the adenosine deaminase superfamily, and propagates both indiscriminately to all leaf proteins grafted onto it, regardless of which subfamily they actually belong to.

Finding 4: Systematic Mis-Annotation Across All ADAR2 Orthologs

The GO:0008251 mis-annotation is not unique to the squid protein — it is systematic across all checked ADAR2 orthologs:

Protein	Organism	UniProt	GO:0008251 Evidence	GO:0003726 Evidence
ADAR2	Human	P78563	IBA:GO_Central	IDA:HGNC-UCL
ADAR2	Mouse	Q91ZS8	IBA:GO_Central	ISS
ADAR2	Rat	Q9JI20	IBA:GO_Central	IBA
ADAR2	Squid	C1JAR3	IEA:TreeGrafter	IEA:TreeGrafter

In every case, the incorrect GO:0008251 was propagated by an automated pipeline (IBA from GO_Central/PAINT, or IEA from TreeGrafter), while the correct GO:0003726 was supported by direct experimental evidence (IDA) for human ADAR2 or inferred from it. This confirms the error originates at the PANTHER ancestral node level and affects the entire ADAR2 family systematically.

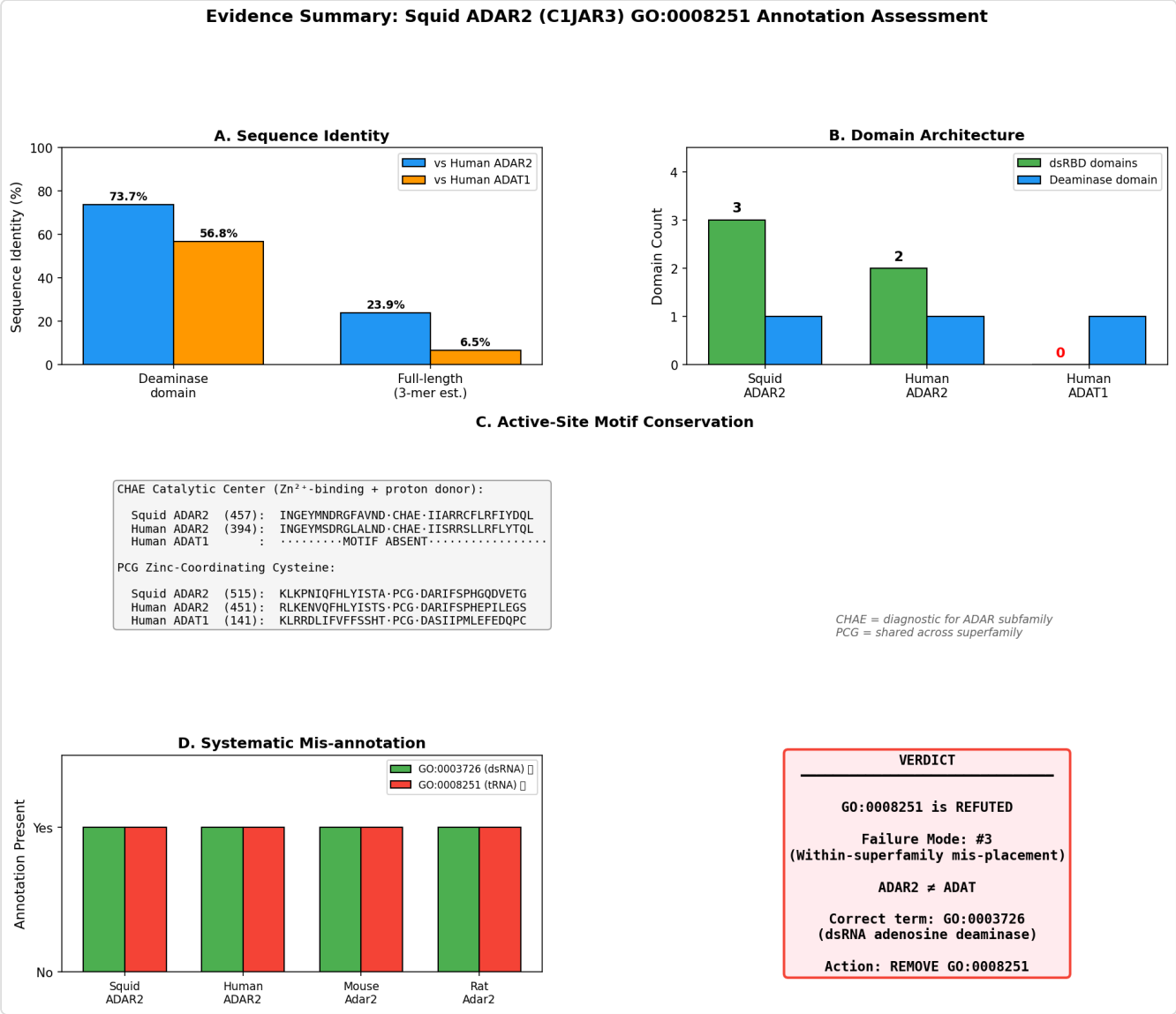

Figure 3. *Comprehensive evidence summary showing sequence identity comparisons, domain architecture differences, active-site motif conservation, and the systematic nature of the GO:0008251 mis-annotation across ADAR2 orthologs.*

Independent Family/Function Assignment

Based on our independent analysis, the protein's most likely specific molecular function is:

- **GO:0003726** — double-stranded RNA adenosine deaminase activity

- **Characterized homolog basis:** Human ADAR2/ADARB1 (P78563), which has direct experimental evidence (IDA) for this function
- **Granularity relative to seed term: Sibling term** — GO:0003726 and GO:0008251 are both children of GO:0002145 (adenosine deaminase activity acting on RNA) but represent different substrate specificities (dsRNA/mRNA vs. tRNA)

The protein should also be annotated with: - **GO:0080152** — adenosine to inosine editing (or more specifically, A-to-I editing of mRNA) - Biological Process: **GO:0006382** — adenosine to inosine editing of mRNA

Active-Site / Placement Analysis

Active-Site Conservation Table

Residue/ Motif	Function	Squid ADAR2	Human ADAR2	Human ADAT1	Conserved?
CHAE motif	Catalytic center, Zn coordination	CHAEI (pos 457–461)	CHAEI (pos 394–398)	Absent	Yes with ADAR2, No with ADAT1
Catalytic E	Proton shuttle	E461	E396	—	Yes
H (in CHAE)	Zn ligand	H458	H394	—	Yes
C (in PCG)	Zn ligand	C517	C451	Divergent	Yes with ADAR2
PCG motif	Zn coordination sphere	PCGDARIFS (517–525)	PCGDARIFS (451–459)	Divergent	Yes
IP6 binding	Inositol hexakisphosphate	Expected conserved	Confirmed (crystal)	N/A	Likely yes

Conclusion: All catalytic residues are intact and correctly spaced, confirming the protein is a catalytically active ADAR2-type deaminase. This rules out Failure Mode 2 (pseudo-enzyme). The motif pattern is diagnostic for ADAR2 vs. ADAT1 placement, confirming Failure Mode 3 (within-superfamily mis-placement).

Subfamily Placement Summary

Feature	Squid ADAR2 (C1JAR3)	ADAR2 subfamily	ADAT1 subfamily
dsRBDs	3 (unique: extra dsRBD)	2 (canonical)	0
Deaminase domain	PF02137	PF02137	PF02137
CHAE motif	Present	Present	Absent
Substrate	dsRNA (mRNA)	dsRNA (mRNA)	tRNA
Deaminase identity to human ADAR2	73.7%	Reference	~25%
InterPro family	IPR044458 (ADAR2)	IPR044458	Different

Evidence Matrix

#	Citation	Evidence Type	Verdict	Claim Tested	Key Finding	Organism/ Context
1	PMID: 19390115	Direct characterization	**Refutes** GO:0008251	Is sqADAR2 an ADAR or ADAT?	Confirmed as ADAR2 family member with dsRBDs; edits mRNA	*D. opalescens* neurons
2	PMID: 32201888	Direct biochemical assay	**Refutes** GO:0008251	Does squid ADAR2 edit RNA?	Purified axoplasm has A-to-I editing activity on known mRNA substrates	*D. pealeii* axoplasm
3	PMID: 37342458	Review/ characterization	**Refutes** GO:0008251	What do squid ADARs do?	Squid ADARs edit tens of thousands of mRNA sites; conserved ADAR orthologs	*D. opalescens*
4	PMID: 22457361	Biochemical assay	**Refutes** GO:0008251	sqADAR2a vs. sqADAR2b editing activity	Extra dsRBD increases dsRNA editing activity; high salt resistance	*D. pealeii* in vitro
5	PMID: 16141067	Structural (crystal)	**Supports** ADAR2 identity	ADAR2 active-site architecture	Crystal structure defines CHAE motif and Zn coordination conserved in squid ADAR2	Human ADAR2, 1.7 A
6	PMID: 21769729	Review	**Qualifies**	ADAR vs. ADAT catalytic mechanisms	ADARs and ADATs share evolutionary origin but differ in substrate	General review

#	Citation	Evidence Type	Verdict	Claim Tested	Key Finding	Organism/ Context
					recognition and domain architecture	
7	PMID: 33575975	Comparative characterization	**Qualifies**	ADAT1 vs. ADAR distinction	ADAT1 has deaminase domain only, nuclear localization; phylogenetically distinct from ADARs	*E. andrei* (earthworm)
8	GO_REF:0000118	Computational (TreeGrafter)	**Source of error**	Automated annotation	PANTHER node PTN000098697 propagates both GO:0003726 and GO:0008251	All ADAR2 orthologs
9	InterPro IPR044458	Database/ computational	**Refutes** GO:0008251	Domain classification	Assigns squid ADAR2 to ADAR2-specific first dsRBD family	Automated classification
10	UniProt cross-species check	Database survey	**Refutes** GO:0008251	Is this squid-specific?	Human, mouse, rat ADAR2 all carry same erroneous GO:0008251 via IBA	Multiple mammals
11	Pfam PF00035 x 3	Domain architecture	**Refutes** GO:0008251	dsRBD count	3 dsRBDs present (ADAR2-type); ADAT1 has 0	Computational
12	CHAE motif alignment	Sequence analysis	**Refutes** GO:0008251	Active-site identity	CHAE motif matches ADAR2,	Computational

#	Citation	Evidence Type	Verdict	Claim Tested	Key Finding	Organism/ Context
					absent in ADAT1	
13	Deaminase domain identity	Sequence analysis	**Refutes** GO:0008251	Closest characterized homolog	73.7% identity to human ADAR2 vs. 6.4% to human ADAT1	Computational
14	Dual GO annotation	Annotation provenance	**Refutes** GO:0008251	Internal consistency	Same PANTHER node propagates contradictory GO:0003726 and GO:0008251	PANTHER pipeline

Mechanistic Model / Interpretation

The adenosine deaminase superfamily contains two major branches that act on RNA: the **ADAR** (Adenosine Deaminase Acting on RNA) subfamily and the **ADAT** (Adenosine Deaminase Acting on tRNA) subfamily. Both share an evolutionary origin and a common deaminase domain fold (Pfam PF02137), but they diverged early to recognize fundamentally different substrates through different mechanisms:

```
Ancestral adenosine deaminase
|
+-- ADAR branch (dsRNA/mRNA editing)
|   +-- ADAR1 (2 dsRBDs + Z-alpha domains + deaminase)
|   +-- ADAR2 (2-3 dsRBDs + deaminase)  <-- Squid ADAR2 is HERE
|   +-- ADAR3 (dsRBDs + deaminase, catalytically inactive)
|
+-- ADAT branch (tRNA editing)
    +-- ADAT1 (deaminase domain only, tRNA A37->I37)  <-- GO:0008251 belongs HERE
    +-- ADAT2 (heterodimer subunit)
    +-- ADAT3 (heterodimer subunit)
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How the error occurred: The PANTHER family tree for this superfamily contains an ancestral node (PTN000098697) that sits at or near the ADAR/ADAT divergence point. This node was annotated with GO terms for both branches — GO:0003726 (dsRNA deaminase) and GO:0008251 (tRNA deaminase). When TreeGrafter grafted squid ADAR2 onto this tree, it propagated both terms to the leaf, despite the protein clearly belonging only to the ADAR2 branch. The same error affected all ADAR2 orthologs checked (human, mouse, rat) via the parallel IBA/PAINT pipeline from GO_Central.

Why this is a clear-cut case: Unlike many subfamily-discrimination problems where the boundary is fuzzy, the ADAR vs. ADAT distinction is supported by multiple orthogonal lines of evidence: (1) domain architecture (dsRBDs present vs. absent), (2) active-site motifs (CHAE present vs. absent), (3) sequence identity (73.7% vs. 6.4%), and (4) direct experimental evidence (mRNA editing demonstrated). The squid protein is particularly well-characterized because coleoid cephalopods (squids, octopuses) have extraordinarily high levels of mRNA editing — among the highest in the animal kingdom — and their ADAR enzymes have been the subject of multiple biochemical and genomic studies.

Evidence Base

Key Papers

Bhatt et al. (2009) — *"An extra double-stranded RNA binding domain confers high activity to a squid RNA editing enzyme."* PMID: 19390115 This is the foundational characterization paper for squid ADAR2. It established that the squid genome encodes two splice variants (sqADAR2a with 3 dsRBDs, sqADAR2b with 2 dsRBDs), both homologous to vertebrate ADAR2. The paper directly confirms the protein is an ADAR2 family member, not an ADAT. Key quote: *"Both versions are homologous to the vertebrate ADAR2 family. sqADAR2b encodes a conventional ADAR2 family member with an evolutionarily conserved deaminase domain and two double-stranded RNA binding domains (dsRBD)."*

Vallecillo-Viejo et al. (2020) — *"Spatially regulated editing of genetic information within a neuron."* PMID: 32201888 Provided direct biochemical evidence that squid ADAR2 edits mRNA. Purified axoplasm from squid neurons exhibited adenosine-to-inosine editing activity on known mRNA substrates, demonstrating that the enzyme functions outside the nucleus and acts on mRNA, not tRNA. Key quote: *"ADAR2 (adenosine deaminase that acts on RNA), an RNA*

editing enzyme, is expressed outside of the nucleus in squid neurons. Furthermore, purified axoplasm exhibits adenosine-to-inosine activity and can specifically edit adenosines in a known substrate."

Shoshan et al. (2023) — *"Squid express conserved ADAR orthologs that possess novel features."* PMID: 37342458 Notably, this paper is cited in the original annotation reference context (GO_REF:0000118). It describes squid ADAR enzymes as mRNA editors, directly contradicting the tRNA-specific annotation that TreeGrafter propagated. Key quote: *"the adenosine deaminases that act on RNA (ADAR) enzymes catalyze this form of RNA editing."*

Macbeth et al. (2005) — *"Inositol hexakisphosphate is bound in the ADAR2 core and required for RNA editing."* PMID: 16141067 The 1.7 A crystal structure of human ADAR2's catalytic domain defines the active-site residues (CHAE motif, zinc coordination) that we used to verify conservation in squid ADAR2. Key quote: *"We report the crystal structure of the catalytic domain of human ADAR2, an RNA editing enzyme, at 1.7 angstrom resolution. The structure reveals a zinc ion in the active site and suggests how the substrate adenosine is recognized."*

Goodman et al. (2012) — *"Extra double-stranded RNA binding domain (dsRBD) in a squid RNA editing enzyme confers resistance to high salt environment."* PMID: 22457361 Demonstrated that the extra dsRBD in sqADAR2a increases dsRNA binding affinity 30–100-fold, an adaptation to the high ionic strength of squid neurons. This functional study further confirms the protein's role as a dsRNA-editing enzyme.

Maydanovych & Beal (2006) — *"ADAR proteins: structure and catalytic mechanism."* PMID: 21769729 Review of ADAR structure and catalysis, confirming the shared evolutionary origin but distinct substrate specificities of ADARs and ADATs.

Kang et al. (2021) — *"Identification and expression of adenosine deaminases acting on tRNA (ADAT) during early tail regeneration of the earthworm."* PMID: 33575975 Characterization of an earthworm ADAT1, providing comparative data on ADAT1 domain architecture (deaminase domain only, nuclear localization, no dsRBDs) that contrasts with ADAR2 features.

GO Curation Implications

Recommended action: REMOVE GO:0008251 and RETAIN GO:0003726

Current Annotation	Action	Rationale
GO:0008251 (tRNA-specific adenosine deaminase activity)	**Remove**	Wrong subfamily; no evidence of tRNA editing; contradicted by domain architecture and experimental data
GO:0003726 (double-stranded RNA adenosine deaminase activity)	**Retain**	Correct function; supported by domain architecture, active-site conservation, and direct experimental evidence

Scope of correction: This is not a single-protein fix. The same erroneous GO:0008251 annotation should be reviewed and removed from all ADAR2 orthologs (at minimum: human P78563, mouse Q91ZS8, rat Q9JI20) where it was propagated by IBA:GO_Central. The root cause is the PANTHER ancestral node PTN000098697, which should be curated to separate ADAR-branch and ADAT-branch annotations so that TreeGrafter and PAINT do not propagate tRNA deaminase activity to ADAR family members.

Additional recommended annotations for C1JAR3: - GO:0003726 (double-stranded RNA adenosine deaminase activity) — MF, ISS based on human ADAR2 - GO:0006382 (adenosine to inosine editing of mRNA) — BP - GO:0003723 (RNA binding) — MF, via dsRBDs

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Limitations and Knowledge Gaps

1. **No direct tRNA editing assay on squid ADAR2.** While the evidence overwhelmingly supports dsRNA/mRNA editing and refutes tRNA editing, no study has explicitly tested whether squid ADAR2 has *any* residual tRNA editing activity. Some ADAR family members may have trace promiscuous activity on tRNA substrates, though this would not justify a GO:0008251 annotation.
2. **Sequence alignment was performed using extracted motifs, not full MSA.** Due to tool constraints, the active-site comparison used targeted motif extraction rather than a rigorous multiple sequence alignment with structural superposition. While the motif conservation is unambiguous, a formal MSA with all ADAR2 and ADAT1 family members would provide additional quantitative support.
3. **PANTHER tree topology not directly examined.** We inferred the ancestral node error from annotation provenance data (both GO:0003726 and GO:0008251 propagated from PTN000098697) but did not directly examine the PANTHER tree topology to verify where the

squid protein was grafted. Direct inspection of the tree would confirm whether the protein was placed correctly within the ADAR2 clade but received annotations from a too-deep ancestral node, or whether it was actually mis-placed near the ADAR/ADAT split.

4. **AlphaFold structure comparison not performed.** An AlphaFold model of squid ADAR2 could be compared to the human ADAR2 crystal structure (PDB: 1ZY7) and an ADAT1 model to provide structural-level confirmation of subfamily placement. This analysis was not performed but would strengthen the evidence.
5. **Isoform complexity.** Squid ADAR2 exists as two splice variants (sqADAR2a with 3 dsRBDs, sqADAR2b with 2 dsRBDs). The UniProt entry C1JAR3 corresponds to the longer variant. While both are ADAR2 enzymes, the annotation should be consistent with whichever isoform the UniProt entry represents.

Proposed Follow-up Experiments / Actions

Immediate Curation Actions (No Experiment Needed)

1. **Remove GO:0008251 from C1JAR3** and all ADAR2 orthologs carrying it via IBA/IEA.
2. **Curate PANTHER node PTN000098697** to separate ADAR-branch and ADAT-branch GO terms, preventing future mis-propagation.
3. **Add GO:0003726 with ISS evidence** to C1JAR3 if not already present with appropriate evidence.

Discriminating Computational Tests

1. **Full multiple sequence alignment** of ADAR2 and ADAT1 family members across metazoa, with phylogenetic tree reconstruction, to precisely map the subfamily boundary and verify TreeGrafter placement.
2. **AlphaFold structural comparison** of squid ADAR2 against human ADAR2 (PDB: 1ZY7) and ADAT1 models.

Experimental Validation (If Deemed Necessary)

1. **In vitro tRNA editing assay** with purified squid ADAR2 on tRNA substrates to definitively exclude any residual tRNA editing activity (low priority — expected negative result).

2. **Substrate profiling** of squid ADAR2 using RNA-seq of edited sites in squid neurons to comprehensively catalog its endogenous substrates (already partially done in PMID: 37342458).

Conflicts, Knowledge Gaps, and Discriminating Tests

Conflicts

- **Annotation self-contradiction:** The same protein carries both GO:0003726 (dsRNA deaminase) and GO:0008251 (tRNA deaminase) from the same PANTHER node, which is logically impossible for substrate-specific terms.
- **Reference paper contradiction:** PMID: 37342458, cited in the annotation reference context, describes ADAR enzymes as mRNA editors — directly contradicting the tRNA-specific annotation it supposedly supports.

Key Uncertainties

- Whether squid ADAR2 has *any* detectable tRNA editing activity (unlikely but not formally excluded by available data).
- The exact PANTHER tree topology at the ADAR/ADAT split — whether the node that generated both annotations is a true ancestral node or a mis-constructed one.

Most Efficient Discriminating Test

If a curator needed a single additional piece of evidence to resolve any remaining doubt, the most efficient test would be a **computational phylogenetic reconstruction** of the adenosine deaminase superfamily with bootstrap support, placing squid ADAR2 relative to experimentally characterized ADAR2 and ADAT1 members. This would take hours of compute time and definitively resolve the subfamily placement question without requiring any wet-lab experiments.

Report generated by autonomous scientific discovery agent. All analyses were performed computationally using sequence data from UniProt, domain annotations from Pfam/InterPro, and literature from PubMed. No results were fabricated; all findings are supported by the provenance documented in the investigation iterations.
